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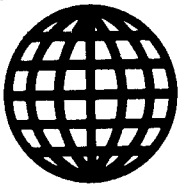

JPRS Report

Science & Technology

USSR: Life Sciences

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UDC 577.133.4:541.127

Complementarily Targeted Modification of Double-Stranded DNA Within Three-Strand Complex

18400466d Moscow DOKLADY AKADEMII NAUK SSSR in Russian Vol 300 No 4, Jun 88 (manuscript received 8 Dec 87) pp 1006-1009

[Article by D. G. Knorre, academician, V. F. Zarytova, L. M. Podust and O. S. Fedorova, Novosibirsk Institute of Bioorganic Chemistry, Novosibirsk Department, USSR Academy of Sciences]

[Abstract] With a view toward eventual targeted genome modification, studies were conducted on targeted modification of double-stranded DNA within a three-strand DNA complex. This approach was selected based on the fact that purine homopolynucleotides are capable of forming specific complexes with two strands of complementary pyrimidine homopolynucleotides. In the present case a 302 nucleotide-long DNA sequence was employed as the target, containing as it did a sequence consisting of 18 guanosine moieties in the 17-34 base position. The DNA target was reacted with either 5'-4[N-2-chloroethyl-N-methyl)amino-benzylamide nano- or pentadecadeoxyribocytidylate in Na acetate buffer, pH 4.0-7.5, at 25°C for 25 h. Following piperidine hydrolysis of the products at 90°C, the fragments were analyzed by electrophoresis in 8% polyacrylamide gel with 7 M urea and autoradiographed. Maximum modification occurred at pH 4.5, i.e., under conditions that are optimal for the formation of the triplex. Modification, as expected, occurred in the G^{17}-G^{34} segment in the double- and single-stranded DNA fragments, and approached 40-50%. The results obtained here provided additional confirmation that site-specific labeling of double-stranded DNA is an attainable goal. Figures 3; references 15: 8 Russian, 7 Western.

UDC 542.91.547.1

Synthesis and Effects on Mammalian Cholinesterases of Bis-(2-Morpholino-1-Methylethyl) Carboxylates

18400474a Tashkent KHIMIYA PRIRODNYKH SOYEDINENIY in Russian No 2, Mar-Apr 88 (manuscript received 14 Jul 87) pp 239-243

[Article by M. Gulyamov, D. N. Dalimov, Z. Tilyabayev, F. G. Kamayev and A. A. Abduvakhabov, Institute of Bioorganic Chemistry, Uzbek SSR Academy of Sciences, Tashkent]

[Abstract] Cursory technical details are presented on the synthesis of the bis-(2-morpholino-1-methylethyl) esters of adipinic, sebacic, azelaic, and glutaric acids and conversion of the esters to the dihydrochloride and diiodomethylate forms for enzymatic studies. The target enzymes employed consisted of human erythrocyte acetylcholinesterase (AChE) and equine serum butyrylcholinesterase (BChE). The resultant derivatives were reversible competitive inhibitors of both enzymes, with hydrochlorides showing less affinity than the iodomethylates. In the case of the dihydrochlorides K_i for AChE fell 84.2-fold in going from n = 4 to n = 8 methylene groups. A similar but less pronounced relationship applied to BChE. The effect of n on K_i was far less pronounced in the case of the diiodomethylates. In summary, the enzymatic studies demonstrated that the hydrochlorides were equally inhibitory for AChE and BChE, whereas the iodomethylates were far more inhibitory for AChE than for BChE. Tables 2; references 11: 8 Russian, 3 Western.

UDC 665.117.4.093.5

Ricinus communis Seed Proteins. Part 4. Amino Acid Sequence of Alanine Subunit of Ricin T of Central Asian Castor Plant: Peptide Maps of Limited Tryptic Digestion

18400474b Tashkent KHIMIYA PRIRODNYKH SOYEDINENIY in Russian No 2, Mar-Apr 88 (manuscript received 25 May 87; in final form 7 Dec 87) pp 243-248

[Article by D. A. Khashimov, Kh. G. Alimov and P. Kh. Yuldashev, Institute of Plant Substance Chemistry, Uzbek SSR Academy of Sciences, Tashkent]

[Abstract] In order to further define the molecular differences among the several forms of ricin, conventional peptide chemistry techniques were employed in an analysis of ricin T isolated from the Central Asian castor plant. Structural studies on the carboxymethylated alanine subunit of ricin T, containing 256 amino acids, involved limited tryptic hydrolysis and chromatographic analysis on Aminex Q-150 and high-voltage electrophoresis on paper. A total of 14 peptides were isolated. The complete amino acid sequences of nine of the peptides were elucidated, with partial sequencing reported for the remaining five peptides. Figures 2; tables 3; references 19: 8 Russian, 11 Western.

UDC 549.953:665.37

Use of Silylation in Synthesis of Fragment 1-4 of ACTH

18400474c Tashkent KHIMIYA PRIRODNYKH SOYEDINENIY in Russian No 2, Mar-Apr 88 (manuscript received 11 Jun 87) pp 248-253

[Article by A. K. Rabinovich and Ye. P. Krysin, All-Union Scientific Research Institute of Blood Substitute and Hormonal Preparation Technology, Moscow]

[Abstract] A novel approach was taken to the synthesis of the 1-4 sequence of ACTH, relying on the use of silylation in the synthetic process for simplification and avoidance of side products. Bis(trimethylsilyl)acetamide and trimethylchlorosilane were employed for silylation,

with ethyl chloroformate used as the condensing agent in the mixed anhydride method. The peptide was obtained in a 60% yield (30.6 g). Melting points, optical rotation, R_f values, and ^{13}NMR shifts for the final product and intermediate peptides are tabulated. Tables 2; references 10: 4 Russian, 6 Western.

UDC 547.96

Synthesis of Putative Aggression Peptides

18400474d Tashkent KHIMIYA PRIRODNYKH SOYEDINENIY in Russian No 2, Mar-Apr 88 (manuscript received 17 Jul 87) pp 253-255

[Article by Ye. I. Sorochinskaya, L. I. Leontyeva and V. F. Martynov, Leningrad State University imeni A. A. Zhdanov]

[Abstract] Synthesis of pyroglutamyl-asparaginyl-glycine was attained by peptide techniques relying on condensation of the benzyl ester of asparaginyl-glycine with the pentafluorophenyl ester of N-carbobenzoxy-pyroglutamic acid, in order to test the tripeptide for its reported aggression-inducing activity. [Reichelt, KL, et al., PSYCHOPHARMACOLOGY OF AGGRESSION, NY, Raven Press, 1979, p. 159]. The tripeptide was tested on outbred mice and rats (0.5-1 mg/kg s.c.; 0.1 mg/25 microliter or 0.25 mg/50 microliter Hanks solution in brain ventricles; 0.2 mg/200 microliter intracardiac) and found to be without effect on behavior. In addition, trials with the structural analog prolyl-asparaginyl-glycine also failed to show any behavioral effects. References 5: 1 Russian, 4 Western.

UDC 547.993

Vasoactive Peptides From Venom of Vespa orientalis: Physicochemical and Functional Characteristics

18400474e Tashkent KHIMIYA PRIRODNYKH SOYEDINENIY in Russian No 2, Mar-Apr 88 (manuscript received 8 Sep 87) pp 255-258

[Article by V. M. Lvov, A. A. Kolmakova, A. A. Akhunov and I. F. Mukhamedov, Institute of Bioorganic Chemistry, Uzbek SSR Academy of Sciences, Tashkent]

[Abstract] Conventional protein methodology was utilized in the isolation of two peptides from hornet (Vespa orientalis) venom that possessed vasoactive properties. Designated peptides I and II, they consisted of 11 amino acids with respective molecular weights of 1100 and 1500 and isoelectric points of 10.65 and 10.50. The myotropic activities of I and II were less pronounced than those of bradykinin, and both peptides were inactivated in this respect by reaction with antibodies against bradykinin. However, whereas bradykin is highly susceptible to destruction by chymotrypsin and kininase II, peptides I and II were much more refractory. Peptide II and bradykinin were essentially equivalent in eliciting hypotension on intravenous administration to cats, while peptide I had to be administered in much higher concentrations. Finally, both peptides were far more efficient than bradykinin in inducing histamine release from mast cells. Radioimmunoassay studies demonstrated that there is considerably more structural homology between bradykinin and peptide II than between bradykinin and peptide I. Figures 2; references 12: 2 Russian, 10 Western.

Hydrodynamic Studies of Aqueous Solutions of Yersinia pseudotuberculosis Lipopolysaccharide-Protein Complex

18400475c Moscow BIOFIZIKA in Russian Vol 33 No 2, Mar-Apr 88 (manuscript received 23 Sep 86) pp 288-292

[Article by I. M. Yermak, G. M. Yadykina, T. F. Solovyeva and Yu. S. Ovodov, Pacific Institute of Bioorganic Chemistry, Far Eastern Scientific Center, USSR Academy of Sciences, Vladivostok]

[Abstract] Viscosimetric studies were conducted on aqueous solutions of a lipopolysaccharide-protein (LPSP) complex isolated from Yersinia pseudotuberculosis to ascertain the size of LPSP particles and the relationship between the intrinsic viscosity and molecular weight. The studies were conducted with a Ubbelohde viscometer at 20.0 plus or minus 0.1°C. At concentrations below 0.7 mg/ml the viscosity vs. concentration plot was linear. The relationship between the intrinsic viscosity and the molecular weight was described by the following relationship: $[\eta] = 5.5 \times 10^{-4} \times M^{0.57}$. In aqueous solution, the LPSP complexes form compact spheres 8 to 14 nm in diameter. Figures 3; references 11: 7 Russian, 4 Western.

Drift Mechanisms of Spiral Wave in Nonhomogenous Medium

18400475d Moscow BIOFIZIKA in Russian Vol 33 No 2, Mar-Apr 88 (manuscript received 11 Nov 86) pp 338-342

[Article by A. M. Pertsov and Ye. A. Yermakova, Institutes of Biological Physics (Pushchino, Moscow Oblast) and of Chemical Physics (Moscow), USSR Academy of Sciences]

[Abstract] A mathematical analysis was conducted on the drift mechanisms of spiral waves in a nonhomogenous medium, resulting in the demonstration that in cases where the period of wave rotation is greater than the refractory period of the medium (T/T_r is much greater than 1) drift occurs perpendicular to the gradient of nonhomogeneity. In situations where ε is much less than 1 (with $\varepsilon = 0$ for homogenous media) the velocity of the drift was expressed by $v = 1/2\varepsilon R_o^3\omega$ for the motion of R(x, y). The trajectory of the drift was described by the coordinates $x = R_o \cos\omega t + 1/2\varepsilon R_o^3\omega t$ and $y = R_o \sin\omega t$. The differences between theoretical data and experimental data obtained on the basis of the Fitz Hugh-Nagumo model were within 10-15%. Figures 3; references 11: 7 Russian, 4 Western.

USSR-CMEA Country Cooperation in Protein Synthesis

18400428 Moscow EKONOMICHESKOYE SOTRUDNICHESTVO STRAN-CHLENOV SEV in Russian No 4, Apr 88 pp 13-18

[Article by journalist Aleksandr Yuskovets, based on an interview with Valeriy Vasilyevich Beregovykh, doctor of technical sciences and director of the All-Union Scientific Research Institute for the Biosynthesis of Protein Substances: "Microbiologists Make the Weather"]

[Text] The end of the twentieth century has gotten us used to the unlikeliest discoveries providing progress. That which not so long ago was considered fantasy is considered commonplace today. Take the field of biochemistry, for example: it turns out that protein—one of the basic nutritional components both for man and for farm animals—can be derived from petroleum, wood, or gas.

Studies have been going on in that area for a quarter of a century now. The result? In the USSR alone, more than one million tons of nutrient yeasts are produced every year from various types of inedible materials—from wood and plant by-products, from ethyl alcohol, and from purified liquid petroleum paraffins. The Mozyr Nutrient Yeasts Plant in Belorussia manufactures food additives from those very sources. That 300,000-ton-capacity enterprise, as we know, was built through the joint efforts of East Germany, the Republic of Cuba, Poland, the USSR, and Czechoslovakia. Because of their contribution to this international structure, the USSR's partners get finished products from Mozyr.

The technology for producing nutrient yeasts was developed at the All-Union Scientific Research Institute for the Biosynthesis of Protein Substances. It is, perhaps, the only institute of its kind today that has comprehensively solved problems involving the creation in an industrial setting of biomass consisting of four types of material—standard petroleum paraffins, ethyl alcohol, methyl alcohol, and natural gas.

For that reason, the institute has become the head organization in handling one of the tasks given priority in the Comprehensive Program of Scientific and Technical Progress (CPSTP)—the accelerated development of biotechnology. In close contact with domestic organizations in the USSR and with colleagues from Bulgaria, Hungary, East Germany, Rumania, and Czechoslovakia, the institute is conducting a series of studies whose aim is to introduce the technology for producing protein-vitamin concentrates from methyl alcohol.

Contributing to the Supply Problem

We met with the director of the institute, Doctor of Technical Sciences Valeriy Vasilyevich Beregovykh, right after his return from the People's Republic of China, where the work of the institute involving modern technologies that use single-cell organisms to produce biomass was, understandably, of great, practical interest.

"By the way," says Beregovykh, "the interest is being shown not only in China. Everywhere, where the problem of providing foodstuffs, especially with protein, is rather acute. In particular, a broad-based theoretical and applied search is also being conducted in that field in highly developed countries like the United States, Great Britain, West Germany, Italy, Spain, and Japan. Is it any wonder that the problem we are working on has one of the highest priorities in the CPSTP?"

"Nevertheless, why such a riveting interest now?"

"Everyone knows that the planet's population growth is requiring a constant increase in the production of foodstuffs and of feed for farm animals. The primary source today and in the near future is agriculture. Nowadays—and specialists are well aware of this—the possibilities for a significant expansion of cultivated land and for a sharp increase in crop yield are becoming increasingly limited. This has to do with providing protein-balanced foodstuffs and feed products."

At the same time, there is a shortage of the traditional forms of protein for use in foodstuffs and feed. A number of countries—because of their geographical location and their environment, soil, and climate—are experiencing certain difficulties with traditional protein additives such as bone meal and soybean oil cake.

The USSR, let's say, fulfills only one-tenth of its needs in feed protein right now. Similar problems exist in the other CMEA member-countries. Naturally, we don't have the right to tolerate such a situation. Only an effective scientific search will help find a way out of the situation that has developed.

There is yet another consideration dictating the need to rapidly introduce biotechnology into the production of protein from inedible components. Many countries are highly dependent on market conditions for soybean products, since soybeans are the principal source for the production of high-grade protein feeds. Some 90 percent of the world soybean production is in the United States. And that, it seems, may also explain the elevated interest of the CMEA member-countries in alternative sources of feed protein.

In turn, the USSR is among the leading country-producers—from a technological point of view—of protein from nontraditional sources. We have accumulated a great deal of scientific reserve and long-term experience in the operation of large industrial installations and plants. At present, the scales of production of feed protein made from standard petroleum paraffins are unrivaled in the world. Overall, a biomass of yeasts grown on petroleum hydrocarbons account for some 70 percent of the protein by volume."

Technological Aspects

"And what, specifically, is the advantage that the new technologies have over, say, the traditional, agricultural techniques?"

"The new direction of the protein industry," Beregovykh continues, "was born nearly a quarter of a century ago, and the interest displayed in it by the CMEA member-countries has existed, as I already said, about 15 years. By the way, our institute has been doing joint work with specialists from East Germany since 1974. As a result, the technology for producing feed protein additives from petroleum has been developed, and the technology for producing protein from gas is being developed. Its name, gaprin, indicates that the yeasts were grown with micro-organisms right on the natural gas.

This is not the first time Soviet scientists have worked with their colleagues from Bulgaria, Rumania, and Czechoslovakia. Therefore, the consolidation of effort in carrying out the CPSTP has been something of a continuation of this joint work.

As far as advantages over the traditional agricultural methods go, they are, first, the high rate of protein synthesis in the cells of the microorganisms—tens of times higher than in plants, and hundreds of times higher than in animal bodies. Second, only the development of the industrial method of production can provide the stable level of production of protein additives in zones of high-risk farming independent of climate, weather, or season, for example. You might say that the microbiologists are making the weather!

It's important to consider this point. An enterprise that manufactures 100,000 tons of microbial feed protein a year requires 40-45 hectares of land. Naturally, it can be built on land that is not suitable for farming.

But how much land must be planted with soybeans or grains to produce that amount of protein? You won't believe it—4.5-8.5 thousand times more!

And there's yet another factor that has economic significance. The production of protein with single-cell micro-organisms on standard petroleum paraffins and other nontraditional substrates, including methyl alcohol, is based entirely on domestic scientific developments in biotechnology.

But can these artificially grown feed additives, which turn up in animal feed, have any kind of harmful effect on the human body later on?

"None at all," Beregovykh announces confidently. "Suffice it to say that in the USSR, protein-vitamin concentrate has been produced for more than 20 years. During that period, more than 2.5 million tons have been produced. Specialists from the USSR Ministry of Health have conducted multiyear tests, and they have shown the absolute safety of these additives. The researchers have established that the material used for the production of the yeasts does not contain carcinogens. Nor have any other negative effects been detected."

To the contrary, lengthy tests on large animal populations have established the high biological value of protein-vitamin concentrates, one that is second to none among the other protein feed types.

How Meprin Is Grown

This is also absolutely true for the technology developed in accordance with the CPSTP for producing meprin—feed additives made from methyl alcohol. What is its essence?

"Feed additives," explains the director of the institute, "depend on the type of material used and are called paprin, of which we already spoke and which is produced on the basis of standard petroleum paraffins; eprin, which is based on ethyl alcohol; meprin, which is based on methyl alcohol, and gaprin, which results from yeast growing on natural methane gas."

The process is the same for all types of material. The solution of nutrient salts and inoculant is first prepared, and then the fermentation begins—the uninterrupted cultivation of the producer-strain, or producer. Later come processes such as heat treatment and drying.

During the joint work, special attention is paid to environmental protection measures. After all, the process consumes a lot of energy, and a great deal of water is required for it. If the cycle is broken, the waste water must undergo special biological purification. A system of closed-loop, repeated use of the water is also called for. The airborne emissions into the atmosphere must be carefully cleaned.

The most complex feature of the technological process is the cultivation of special bacterial strains on methyl alcohol. It must be said that, unlike the strains produced by, say, the English firm Imperial Chemical Industry, our strains are more resistant to outside flora and do not require sterile production conditions. And that, of course, has an economic advantage.

Industrial producer-strains are taken from nature and are chosen on the basis of a number of properties. They have the highest content of unsubstituted amino acids, especially those in which plant and grain products used in feed production are deficient—specifically, lysine and sulfur-containing amino acids.

Among the other merits are ease of extraction from the culture medium and the absence of toxicity and other harmful effects on the body of the animal and man.

The development and operation of high-volume production of feed protein and the accelerated rates of growth of microbiological, biochemical, and technological research in yeast production have created a base for the development of new trends in biological machine-building. Today there are no technical roadblocks to the building of powerful biotechnological combines capable of producing 200,000 tons of feed protein a year. High-mass-exchange fermenters developed by Soviet specialists and already in use produce more than 40 tons of biomass a day. That is far greater than what is produced by the other units used around the world.

The joint work of the scientists from the CMEA member-countries involves modernizing the equipment; creating high-capacity fermenters that ensure the highest coefficient of use of the gaseous and liquid components of the nutrient media; lowering the total energy expense; and ensuring high operational reliability no matter what material is used. This requirement is in the CPSTP. New designs are being developed through the use of computer-aided design and analysis of fermenters.

Specifically, the task of optimizing the growth of the strains has been assigned to the All-Union Scientific Research Institute for the Biosynthesis of Protein Substances. Highly efficient computerized monitoring and control is used for this, as is real-time microscopy. The industrial use of this system has shown that it both raises the productivity of the equipment and saves on raw material, with guaranteed quality of the finished product.

"Now," says Beregovykh, "we and our colleagues from East Germany have more or less completed the scientific studies of the experimental batches of the bacterial biomass made from natural gas and from methyl alcohol. It has been proven that the feed protein products derived as a result of microbiological synthesis are effective feed additives that are harmless. In terms of nutritional properties, for example, paprin compares favorably with high-grade feed proteins of animal and plant origin; meprin shows similar indices.

In addition, the use of microbial protein for nutritive purposes has prospects. The medical and biological characteristics of such proteins enables us to examine the possibility of creating analogs of traditional food. Several variations have been developed for the production of food protein isolates made from various kinds of biomass. The technology for producing food protein assumes the comprehensive refinement of the microbial mass, with the extraction of products that have a completely independent significance. They are amino acids, peptides, coenzymes, nucleic components for later refinement into special products for medical purposes, and microbial lipids. This technology may be based not only on ethyl alcohol, but also on methyl alcohol (the development of which was begun within CPSTP).

A Project of Joint Construction

As calculations show, the creation within the Soviet Union of large-scale, joint production of meprin—which will be used by Bulgaria, Hungary, East Germany, Rumania, the USSR, and Czechoslovakia according to degree of participation in the program—will provide the greatest advantages. This is due to the availability in the Soviet Union of a material base—natural gas, from which methyl alcohol is synthesized—and the nearness of the production to the material source, especially important since the reserves of petroleum, from which standard paraffins are extracted (paprin is grown on them in particular), are steadily declining. The choice rests on natural methane because the technology of its use is virtually complete (joint development with East German specialists).

"Who else is participating in the collaboration?"

"The research associates from the All-Union Scientific Research Institute of Genetics (Moscow) and the Institute of Biotechnology (Leipsig, East Germany), for example, are, with Rumanian specialists, conducting genetic and genetic-engineering research. Working on general problems are the Bulgarian Central Institute of Chemical Industry, the Hungarian enterprise Khemimash, the East German Chemical Installations Combine, the Institute of Chemical and Pharmaceutical Research (Rumania), the production association Khepos in Brno (Czechoslovakia), and the Institute of Microbiology of the Czechoslovakian Academy of Sciences. The combination of the efforts of such powerful collectives is already bringing results. There was never a period of organizational shakiness, all problems are solved quickly, and certain tasks are carried out ahead of schedule."

Of course, every country has experience. Our Czechoslovakian technologists, for example, are well-versed in fermentation chemistry. East German specialists are actively collaborating in programs for the production of a protein mass from petroleum distillates, from gas, and from methyl alcohol. In the USSR, microbiological developments stand at the highest level.

Tests of an experimental industrial installation (capable of producing 10,000 tons of biomass a year) were begun last year in the USSR, and a working design of basic production begins in 1989.

"Judging from the general interest in achieving the final result," concludes Beregovykh, "its start-up will take place on schedule. And when it happens, we will be able to say that microbiologists have actually begun to make the weather: getting high-quality protein additives "to the table" of farm animals will largely cease to depend on the whims of nature, climate, or season. High-quality protein masses will be produced in fermenters."

UDC 632.937.15.002.2:579.842.11.083.13

Improved Biotechnology of Dendrobacillin Production

18400481b Moscow BIOTEKHNOLOGIYA in Russian Vol 4 No 2, Mar-Apr 88 (manuscript received 20 Oct 86) pp 213-216

[Article by E. I. Perov, L. Yu. Zamytskiy, K. B. Alibekov, V. G. Churbanov, V. N. Bugreyev and V. A. Rumyantsev, Progress Plant, Stepnogorsk, Tselinograd Oblast]

[Abstract] An assessment was conducted on the production of dendrobacillin through improvements in the cultivation regimen of Bacillus thuringiensis. The approach was based on the "fed batch culture" method, which was demonstrated to improve bacterial yields through batch addition of carbon and nitrogen substrates. Studies with B. thuringiensis var. dendrolinus 49-8 grown at 28-30°C, 175 rpm, and aeration of 800-1000 m^3/h, with the addition of yeast hydrolysate as indicated by monitored pH and eH values, led to a 2.3-fold improvement in yield over conventional methods of cultivation. The spore concentration in the final products was on the order of 8-9 x 10^9 per ml. Direct drying without preconcentration diminished losses and rendered the process virtually waste-free. The spore preparations showed biological activity equivalent to that of commercial preparations without loss of activity after a year of storage. Implementation of the fed batch culture method in the production of dendrobacillin is expected to be cost-effective on the order of three million rubles per year. Tables 3; references 6: 3 Russian, 3 Western.

UDC 663.14.012.3

Bioenergetic and Instrumental Aspects of Energy Efficient Fermentation Systems

18400481c Moscow BIOTECHNOLOGIYA in Russian Vol 4 No 2, Mar-Apr 88 (manuscript received 20 May 86) pp 235-245

[Article by U. E. Viyestur, Yu. E. Shvinka and M. A. Rikmanis, Institute of Microbiology imeni A. Kirkhenshteyn, Latvian SSR Academy of Sciences, Riga]

[Abstract] A broad discussion is presented of the factors that affect the energy efficiency of bioreactors, using several concrete examples in the case of different fermentation systems. The fundamental approach relies on the design of systems that ensure efficient utilization of the free energy of the substrate. The latter is best assured by relying on bioreactor processes that take advantage of physiological potentials of the substrate to control hydrodynamic parameters, as well as heat and mass exchange. From the technical viewpoint aerobic processes may be most conveniently controlled by the K_{vp} factor (Q_{ox}, kg O_2/m^3). Optimization of the aeration conditions, mixing, and reactor design on the basis of the physiological potential of the substrate being processed should lead to a new generation of more efficient bioreactors. Figures 7; references 30: 17 Russian, 13 Western.

UDC 632.937.16

Developments and Perspectives in Application of Entomopathogenic Viral Preparations in Soviet Economy

18400481e Moscow BIOTEKHNOLOGIYA in Russian Vol 4 No 2, Mar-Apr 88 (manuscript received 19 Aug 88) pp 267-272

[Article by N. A. Bozhko, All-Union Scientific Research Institute of Molecular Biology, Novosibirsk Oblast]

[Abstract] A survey of Soviet efforts in biological pest control have shown them to be quite inadequate and to lag far behind similar programs in the West. In the USSR considerable reliance is still being placed on chemical means with their adverse ecological impact, while biological projects are limited to bacterial entomopathogenic preparations. The time has come to encourage the development of Soviet viral preparations for the control of insect pests and to overcome shortcomings that tend to discourage such research due to material and/or financial problems. One of the most promising directions in encouraging a viable Soviet effort at viral insect control is based on baculoviruses, since experience gained in the West can be used as a starting point for similar research in the USSR. A full-fledged and well-supported research effort will require extensive studies on appropriate tissue culture methodology for viral propagation, the development of insect breeding programs, and effective testing methodology. The biotechnological industry will also be faced with the demand for viral preparations with a shelf-life of 1.5-2.0 years, improved stability on storage that exceeds present performance 2- to 3-fold, and reasonable prices vis-a-vis other insect control options. Tables 2; references 13: 10 Russian, 3 Western.

UDC 614.7:663.1

Industrial Microbiology and Environmental Protection

18400481d Moscow BIOTEKHNOLOGIYA in Russian Vol 4 No 2, Mar-Apr 88 (manuscript received 12 Jun 86) pp 254-261

[Article by M. M. Shapiro, Medbioekonomika Scientific Industrial Association, Moscow]

[Abstract] The input of industrial microbiology to environmental protection finds manifestation in three basic forms of activity. In one category are activities involved in microbiological processes, encompassing production processes and control of inadvertent release of engineered microbes. The second aspect deals with the use and application of products produced by the microbial industry, particularly its biotechnological component. Finally, the last category is concerned with the use of microbial means of environmental protection. Within the industry itself primary emphasis is being currently placed on the implementation of low-waste or, preferably, waste-free technologies through the use of closed systems. Through exercise of pollution control within the various branches of the microbiological industry and via the use of microbiological means of pollution control at other industries, significant abatement of environmental pollution in the USSR can be expected by 1990. The expectation is that by 1990 discharge of pollutants may be reduced by 30.3 percent in comparison with 1986, including a 44 percent reduction in the discharge of polluted waste waters and a 16.6 percent decrease in air pollutants. References 7 (Russian).

UDC 575.13:577.21

Mapping of Replication, Maintenance, and Mobilization of Broad Host Range R Plasmid pBS222

18400484a Moscow GENETIKA in Russian
Vol 24 No 3, Mar 88 (manuscript received 20 May 87) pp 405-413

[Article by B. V. Polevoda, L. K. Gribanova, V. I. Ugarova, A. N. Lebedev, T. V. Tsoy and A. M. Boronin, Institute of Microbial Biochemistry and Physiology, USSR Academy of Sciences, Pushchino, Moscow Oblast]

[Abstract] Mapping studies were undertaken in the broad host range R plasmid pBS222 to define genes responsible for replication, maintenance, and mobilization. A series of restriction and cloning studies followed the induction of deletion mutations by sodium bisulfate and led to identification of virtually identical deletions, suggesting that pBS222 contains recombinational "hot points." At such hot points any structural changes can be expected to result in a recombinational event. The end results of such events may be expected to possess the minimum genetic information required for replication and maintenance. Use of small DNA fragments on the order of 1 to 3 kb made it possible to reduce the fragments to gene-size segments, since the genes of plasmids with a broad host range are, as a rule, scattered over the entire genome. Three derived plasmids were obtained—pBS359 (4.2 kb), pBS361 (7.1 kb), and pBS362 (5.1 kb)—which are among the smallest plasmids shown to retain a broad host range encompassing E. coli and various species of Pseudomonas, and which may be used to construct vectors with a wide host range. In addition, three polypeptides have been identified with molecular weights of ca. 15,000, 25,000, and 30,000 D. The former two entities may be involved in the inheritance of recombinant plasmids in various host species, and the latter peptide may be required for plasmid mobilization. Figures 4; tables 2; references 23: 5 Russian, 18 Western.

UDC 575.113:579.8

Effectiveness of Wheat Lr Genes and Their Combinations Against Brown Rust Pathogens

18400484b Moscow GENETIKA in Russian
Vol 24 No 3, Mar 88 (manuscript received 28 Jan 86; in final form 7 Aug 86) pp 510-517

[Article by M. Ye. Sinigovets, All-Union Scientific Research Institute of Phytopathology, Moscow Oblast]

[Abstract] An analysis was conducted on the effectiveness of the various Lr genes (Lr1 to Lr26) of wheat offering resistance against the various races of the brown rust pathogen prevalent in the USSR. The plant infection studies were evaluated on the basis of the Cobb scale, with the susceptibility studies combined with breeding studies conducted in 1981-1985. The studies conducted under the conditions prevalent in the Moscow region at temperature of 15-20°C, 18-22°C, and 20-28°C showed that with the exception of gene Lr22b, all of the Lr genes afforded protection against one or more races of the pathogen. In particular, genes Lr9, Lr19, Lr21, and Lr24 were rated as imparting resistance or moderate resistance (Cobb scale) against all of the pathogenic races employed in the study. Gene Lr18 was found to be most temperature dependent for its effectiveness, and genes Lr2b and Lr13 offered protection against relatively avirulent pathogens at all stages of plant development. These observations constitute a foundation for breeding studies designed to provide Lr gene combinations offering the greatest degree of protection against wheat loss due to brown rust. Tables 3; references 21: 5 Russian, 16 Western.

Properties of Staphylococcal Enterotoxin B and Its Segment Similarity to Thymopoietin II

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[Article by R. E. Vegner, I. R. Rituma, L. U. Podinsh, and G. I. Chipens, Red Banner of Labor Institute of Organic Synthesis of the Latvian Academy of Sciences]

[Text] A model has been proposed for studying the molecular mechanisms of the immune response[1] in which low-molecular peptide immunoregulators form from high-molecular-weight precursors (such as thymus hormones, immunoglobulins, interleukins, and proteins from the main tissue compatibility complex) during their interaction with lymphoid cells in reactions of limited proteolysis. When their chemical structures are being ascertained, one methodological approach, based on the concept of biochemical universality, is to compare the amino acid sequences of various proteins that affect the immune system and to identify in their structures the common identical and similar (equifunctional, bearing the same signatures) segments. The use of that approach enabled us to identify a new active center in the staphylococcal enterotoxin B (SEB) molecule and to establish the chemical structure for the first representative of the peptides of the polarine group (Ser-Lys-Asp), which are built primarily from polar amino acids and have a broad spectrum of biological action in the immune system and the central nervous system.

This paper summarizes the literature data on the physicochemical, chemical, and biological properties of SEB and introduces the results of a comparison of the primary structures of SEB and thymopoietin II. Particular attention is paid to the immunological properties of SEB and to the structure-function relationship in its molecule. Comprehensive data on SEB and other staphylcoccal enterotoxins can be found in the pioneering works and surveys of Bergdoll et al.[2-4] and in work done by other researchers [5-8].

SEB is a representative of a group of related proteins and enterotoxins that are produced by certain strains of the bacteria *Staphylococcus aureus.* Enterotoxins are responsible for staphylococcal food poisoning in man and in higher animals. Based on antigenic properties, staphylococcal enterotoxins may be divided into several serological types, designated in chronological order of their identification by the letters of the alphabet[9]. Thus far, serotypes A, B, C, D, E, and F (designated SEA, SEB, SEC, etc.) have been identified. Type C has subtypes C_1-C_3.

In spite of the differences in antigenic properties, the enterotoxins have externally similar enteropathogenic effects. When enterotoxins enter the stomach in quantities of micrograms, or with intravenous injection of even smaller quantities, they cause nausea, vomiting, catarrh of the gastric mucous membrane, and inflammation of the small intestines, or enteritis. General malaise sets in 2-6 hours after they enter the body, lasting from several hours to a day and passing, as a rule, relatively easily. Enterotoxins A and D are encountered most often in food poisoning. Types B, C, and E are also found in food intoxication, and they also often accompany staphylococcal infections and purulent processes[10]. Considerable differences exist in species sensitivity to the nausea-creating action of staphylococcal enterotoxins: man and the rhesus monkey show an acute reaction. The emetic response is weak in pigs, dogs, and cats; it is absent in rodents. Enterotoxin-induced diarrhea in rats, including that caused by SEB[11], may be partially due to a disturbance of the water-salt metabolism and resorption of water from the intestine. Electron microscope data indicate that enterotoxins in large doses (on the order of 150 μg) cause degeneration of the mitochondria of the epithelial cells of the intestine[12]. Very large intravenous doses of SEB (about 1 mg/kg) cause death in experimental animals (monkeys) as a result of functional disturbances and pulmonary edema[13].

Of all the types of enterotoxin, SEB has been studied in the greatest detail, because the staphylcocci release enterotoxin B into the culture liquid in relatively large amounts (up to 500 μg per milliliter of culture) in the laboratory. A prototype of the strain *St. aureus* that produces SEB is a strain that was isolated from the feces of a child hospitalized in 1954 in Washington with a diagnosis of acute nonspecific diarrhea[9]. One and the same strain of bacteria can produce two or more types of enterotoxin. The use of pulse technology for producing a labelled methionine residue [^{35}S] has shown that SEB is produced from a protein precursor associated with the bacterial membrane, is accumulated, and is temporarily stored in special regions of the cell wall[14].

Characteristics of SEB. The first representative of the SEB enterotoxins was purified in 1965 via a combination of several varieties of ion-exchange chromatography[15]. Separating the proteins that accompany SEB and that have almost the same antigenic properties and similar physicochemical properties presents considerable difficulties even for high-performance liquid chromatography[16]. In addition, during growth and purification, partial modification (deamidation) of enterotoxin B is possible.

SEB and the other enterotoxins that have been studied are relatively low-molecular-weight simple proteins. They are hygroscopic and dissolve easily in water and in saline solutions. Enterotoxins are heat resistant and even withstand brief boiling in aqueous solution. SEB is stable in a wide range of pH values—from pH 3 to pH 11. Even the acidity of the gastric juices, which are an effective barrier against many toxic products, do not destroy SEB[2]. Stability varies in relation to chemical denaturation in the different types of enterotoxin. In an 8 M solution of urea, the protein chain of SEA unfolds 50 times faster than in SEB or SEC[17]. Judging from physicochemical and serological properties, SEB has a structure similar to that of SEC, and SEA has a structure

similar to that of SEE; the possibility, however, of the presence of homologous segments among all representatives of the family of enterotoxins should not be excluded.

Primary structure of enterotoxins. The complete amino acid sequences of SEB[18] (Figure 1) and SEC_1[19] have been published, as has the amino acid sequence of SEA[20]. SEB is a single-chain protein that consists of 239 amino acid residues. Its molecular weight is equal to 28,366 daltons[3, 18]. The amino acid sequence of SEB is homologous to that of SEC_1. Marked similarity is found at the C-terminus of both molecules. Of the last 67 amino acid residues, 55 are identical. Many residues of lysine, asparagine, glutamine, and tyrosine are found in the composition of SEB and SEC_1 molecules. The ratios of polar to nonpolar amino acids in SEB and SEC_1 are 134:105 and 131:108, respectively. While the distribution of acidic amino acids in the vicinity of polar and nonpolar amino acids is close to random in the SEB molecule, the polar environment is somewhat preferable for the essential amino acids. Of 42 residues of essential amino acids (arginine, lysine, histidine), 16 are located in the centers of polar tripeptides, 6 are found in the centers of nonpolar tripeptides, and the rest are in a mixed environment.

Secondary structure of SEB. Analysis of the CD and ORD spectra of native SEB shows the presence in the secondary structure of roughly 38 percent β-chains and 9 percent α-helices [ref. 21]. This corresponds to the prediction of secondary structure from amino acid sequence according to Choi and Fasman[21] and other authors[22]. Of interest is the prediction of the localization of reverse turns in the SEB molecule (Figure 1). Reverse turns are usually located near the surface of the protein globule and can be part of functionally active portions and contain bonds accessible to enzymatic cleavage.

The coefficient of electrostatic interaction for the SEB molecule, computed from the data of acid-base titration, does not vary in a wide range of pH values. One may conclude from this that the SEB molecule must have a compact structure, in spite of the large quantity of polar groups usually facing the solvent side. The estimated average radius for the proposed globular form of the SEB molecule is equal to 20.7 angstroms[23].

Of 21 tyrosine residues, only 6 are titrated; those 6 are apparently exposed, and the rest participate in the formation of a hydrophobic molecular core and interact strongly with hydrophobic groups and some polar groups. At pH 11.5, by the corresponding dissociative transition of the phenol group of tyrosine, an alteration in the conformation of the SEB molecule is observed[23].

Enzymatic stability of enterotoxins. Enterotoxins B and C_1 are extraordinarily sensitive to the action of trypsin. The tryptic hydrolysis of SEB after the reduction of the disulfide bond and the carboxymethylation of the cysteine residues leads to cleavage of the Lys^{97}-Thr^{98}bond that is located in one of the predicted reverse turns. The products remain aggregated in one particle, but can be separated with denaturizing agents[24]. If intact, unreduced SEB is exposed to the action of trypsin, then the fragments are held in place with a disulfide bridge[25].

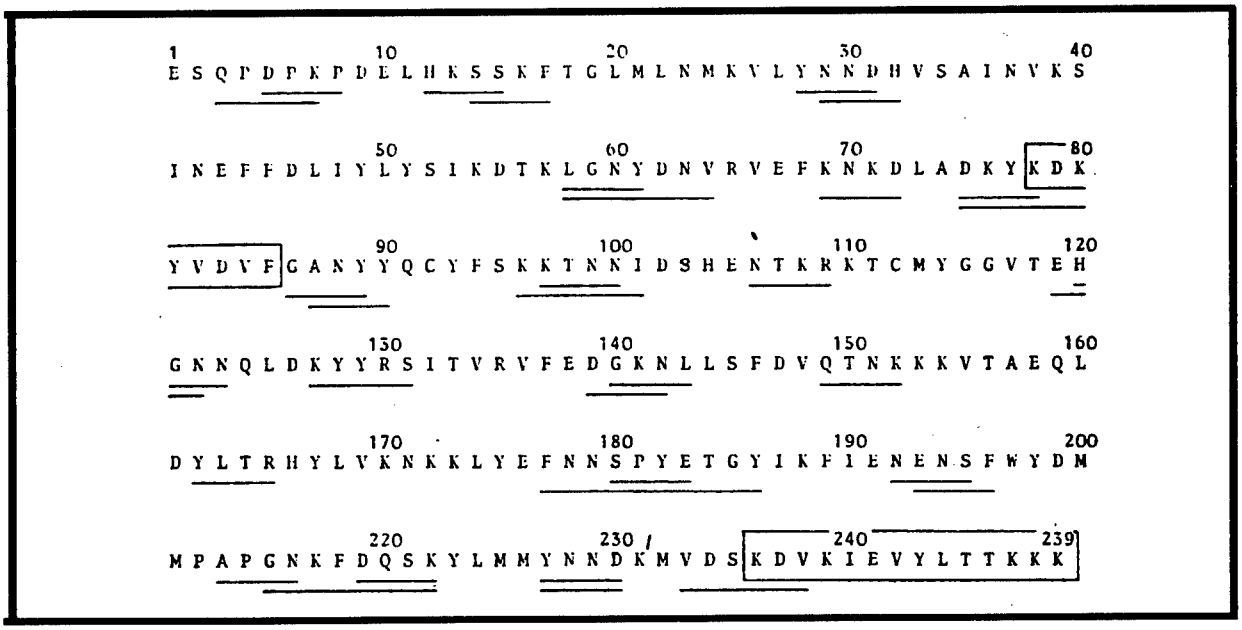

Figure 1. Amino acid sequence of staphylococcal enterotoxin B[18]. Underlined are the segments of the possible localization of reverse turns predicted according to Choy and Fasman[21] (upper lines) and the predictions averaged from five different methods[22] (lower lines). The enclosed segments are those that are homologous to the corresponding segments of thymopoietin II (see Figure 2)

In native SEC_1, of the total number of 32 Lys-X bonds, trypsin can be used to selectively hydrolyze four bonds located at the N-terminus and in the middle part of the protein chain (57-58, 59-60, 98-99, 103-104)[19]. Unlike SEB and SEC_1, enterotoxin A, which has greater epidemiological value, is completely resistant to the action of trypsin, but is nonetheless broken into four fragments by papain[26].

A proteolytic activation mechanism is known for toxins of bacteria that cause diphtheria and botulism. However, for enterotoxins B and C_1, despite the enzymatic lability of certain peptide bonds in these molecules, such a mechanism of action has not been proven[27].

Antigenic properties. Individual types of enterotoxins, according to definition, are serologically specific. However, producing rigorously specific antibodies is a difficult task, because in addition to specific antigenic regions, enterotoxins also contain minor common antigenic regions. Enterotoxin B, for example, can induce in rabbits not only specific antibodies, but also antibodies that react with structurally similar enterotoxins B, C_1, and C_2[28]. The common antigenic regions responsible for cross-reaction are concentrated in the N-terminus of the enterotoxin molecules[29]. Hybridoma technology has been used to produce monoclonal rodent antibodies that selectively interact with enterotoxin B, enterotoxins from group C, and fragments of their tryptic cleavage[30]. With an antiserum against the structurally more distant SEA, no cross-reaction is observed with SEB or SEC_1[31].

Immunological properties. Mitogenic activity. Enterotoxins, including SEB, are highly active polyclonal mitogenic agents of human and animal lymphocytes[32]. The mitogenic activity of SEB is preserved after its nausea-creating properties are removed with formalin[33]. Consequently, different sectors of the SEB molecule are responsible for its toxic properties and for its mitogenic properties[33]. A study of the biological activity of the fragments of the limited enzymatic cleavage of the SEC_1 molecule leads to the very same conclusion[34].

Normal lymphocytes of the lymphatic nodes of a guinea pig exposed to SEB develop a factor that inhibits the migration of macrophages[35]. Enterotoxins (SEA, SEB) in infinitesimally small hormonal concentrations induce the production of α and γ interferons in lymphoid cells[36, 37].

Enterotoxin receptors. The dose/mitogenic effect relationship and the inhibition of mitosis by a specific antitoxin indicate that the polyclonal activation of lymphocytes by enterotoxins A, B, and C is associated with the strong interaction between enterotoxins and the receptors on lymphocytes. All three types of enterotoxin have roughly equal mitogenic activity, from which it follows that the enterotoxin receptors are specific for nonantigenic regions of the molecules[38]. In agreement with such a conclusion is the fact that surplus SEB and SEE rival each other in inhibiting the bonding of labelled [^{125}I] SEA with mouse spleen cells[39].

Effect on T-cell immunity. SEB activates the population of T-suppressor cells (with the phenotype Lyt-1^-, 2^+, 3^+), without macrophages or B-cells participating in the process[40]. Inhibitors of prostaglandin synthesis suppress the effect of SEB on T-suppressors. Apparently, the inducement of T-suppressors depends on the activity of prostaglandin synthetase[41]. In vivo, SEB in relatively large doses (150 µg per mouse) suppresses transplant rejection[42]. This is in agreement with experiments on the level of cells in which SEB activates T-suppressors.

Effect on antibody production. SEB suppresses antibody production[42, 43], which, as with the suppression of transplant rejection, may be due to the action of SEB on T-suppressor cells.

Comparison of the immunological activity of SEB and SEA. When C57BL/6 mice were injected with small doses of enterotoxins in a comparative study of the enterotoxin activity of the 5 serological types A, B, C, D, and E in an experiment involving antibody formation, only SEA and the enterotoxin similar to it in amino acid composition, SEE, substantially reduced the antibody response to sheep erythrocytes[44]. In monkeys who had received an intravenous dose of 0.5 µg/kg of SEA after a period of suppressed immunity that had lasted nearly 24 hours, the SEA caused suppression of the highly active peripheral lymphocytes that rapidly assimilate [^{3}H]-thymidine. Hematological changes responsible for the immune stimulation remained longer than the clinical symptoms of the effects of SEA and, possibly, were mediated by intensified production of interferons and other lymphokines[45]. Unlike SEB and SEC_2, enterotoxin A substantially intensifies the nonspecific resistance of mice to microbial infection[46]. A comparison of the primary structures of SEB and SEA could possibly lead to a prediction of the active regions of SEA that are responsible for the intensified resistance to infection, if the primary structures of SEB and SEA were so similar that sufficiently small segments of SEA could be isolated.

Structure-activity relationship of enterotoxin fragments. SEB in which the peptide bond (97-98) in the segment connected by the disulfide bridge is cleaved by trypsin has full biological activity—serological, mitogenic, and enterotoxic[27]. After the destruction of the disulfide bridge, neither fragment 1-97 nor 98-239 shows any entertoxic activity in rhesus monkeys. Recombinant material that consists of both fragments noncovalently bound acquires the activity of the original protein[24]. This indicates the high complementarity of both fragments and coincides with the notions on the interaction of β-chains in the formation of the three-dimensional structure of the SEB molecule.

When SEC_1 is exposed to trypsin for a lengthy period, two fragments form—an N-terminal fragment 1-59 and a "notched" fragment 60-239, which is joined by the disulfide bridge. The N-terminal fragment shows mitogenic activity and is devoid of enterotoxic activity. On the other hand, the second, larger fragment induces diarrhea in monkeys but does not show any mitogenic activity[34]. Initially, we assumed that the region associated with the disulfide bridge (92-112) was responsible for the enterotoxic activity in the SEB molecule. However, in SEC_1, which also shows enterotoxic activity, the magnitude of the cyclic part of the molecule was smaller than in the SEB by three amino acid residues. This caused us to have misgivings about the correctness of the initial assumption. Our doubts were further substantiated because a trypsin-treated SEC_1 analog in which the 99-103 fragment is absent shows full biological activity[47].

SEA that is incubated with papain forms four fragments that are devoid of entertoxic properties. Only two of the four fragments are associated with interferon-producing and mitogenic activity[26].

Chemical modification of SEB. The amino and carboxylic groups, the disulfide bridge, and the side chains of the methionine and tyrosine residues undergo chemical transformation in the SEB molecule[3]. The guanidination and nitroguanidination of a considerable number of lysine amino groups (by 3,5-dimethyl-1-guanylpyrazole and its nitro derivative or O-methylisourea) does not lead to substantial alterations of the molecular conformation, antigenic activity, or biological activity of SEB[48, 49]. If 32 lysine residues (of a total of 33) were transformed into homoarginine residues, roughly 90 percent of the enterotoxic activity is lost. Apparently, if the lysine side chains are part of the antigenic and toxic portions of the SEB molecule, then rigorous stereospecificity of reception is absent for the overwhelming majority of the lysine residues. Acetylation of the amino groups in SEB (and in SEC) leads to serious conformational alterations of the molecule and to toxin inactivation, which indicates the essential value of the positive charges of the protein chain in the stabilization of molecular conformation and in the manifestation of biological activity.

Modification of up to 24 carboxyl groups (of a total of 33) by means of condensation with glycine methyl ester in the presence of a water-soluble carbodimide has an extremely weak effect on the antigenic and enterotoxic properties of SEB. And only after the blocking of more than 30 carboxyl groups (in the presence of the denaturing agent, guanidine hydrochloride) does the protein chain of the SEB molecule unfold and the enterotoxic activity disappear[50].

The reduction of the disulfide bridge and the alkylation of the SH groups with iodoacetamide or iodoacetate does not lead to loss of biological activity or to a substantial change in the physicochemical properties of SEB—viscosity and sedimentation parameters. This indicates that the native conformation of the entire SEB molecule and its biologically active regions are maintained after the destruction of the disulfide bond[51].

Iodoacetic acid alkylation and hydrogen peroxide oxidation of more than 6 of the 8 side chains of methionine in SEB leads to the loss of enterotoxic activity and, according to fluorimetric analysis, also to alteration of the conformation of the molecule[49].

Similarity of SEB segments and thymopoietin II segments. Analysis of the homology of unrelated proteins that affect one and the same body system—in this case, the immune system—is of interest in the study of the mechanism of action of the compounds we are examining and in the identification of biologically active protein fragments. In searching for a structural similarity between thymus hormone molecules and the molecules of other immunologically active peptides and proteins, as already noted, we identified a similarity between the C-terminal sequences of SEB and of bovine thymopoietin II. In order to achieve the highest correspondence of C-terminal segments (of the 14-component SEB and the 17-component thymopoietin II), three deletions were introduced into the SEB segment. As a result, 8 pairs of identical amino acids and 4 pairs of homologous amino acids were produced in the segments (Figure 2).

The thymopoietin sequence included part of the active center (segment 32-36) and the adjacent region of additional bonding with the thymopoietin receptor (segment

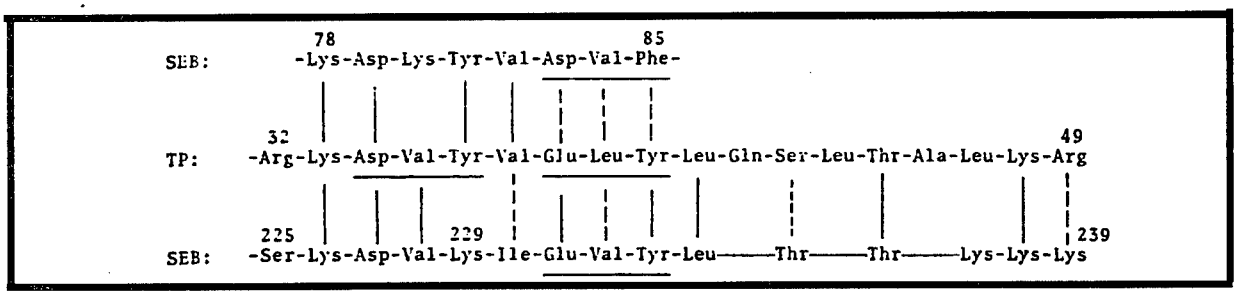

Figure 2. Comparison of the homologous segments of staphylococcal enterotoxin B (SEB)[18] and thymopoietin II (TP)[52]. Identical or potentially equifunctional amino acid residues are joined by vertical solid lines and vertical dashed lines, respectively. Underlined are the segments that consist of residues of acidic, hydrophobic, and aromatic amino acids which are encountered twice in the SEB and TP structures

37-45)[53]. The active center of thymopoietin and the pentapeptide SEB 225-229 that corresponds to it have an identical central tripeptide (Lys-Asp-Val) and differ sharply from each other in terms of the properties of their terminal amino acids, which may account for the competitive antagonism of SEB and thymopoietin II in immunological reactions. Supporting such an assumption is the homology of segment 37-41 of the region of additional bonding of thymopoietin II and its equivalent in SEB—segment 230-234.

Computer analysis of the structures of SEB and thymopoietin II has identified another two homologous 8-component sequences: SEB 78-85 and thymopoietin 33-40 (Figure 2). Interestingly, tripeptides with an acidic-hydrophobic-aromatic amino acid sequence are encountered twice in the SEB and thymopoietin II structures (and only in the homologous sectors). In six of eight instances, tripeptides are located in the vicinity of hydrophobic amino acids (Val, Ile, Leu), which resembles the arrangement of common fragments of the third type in the structures of immunoregulators and other proteins[1].

Since SEB is quite similar in structure to SEC_1, the corresponding segments of SEC_1 and thymopoietin II are also homologous, although, in this case, the homology is somewhat weaker.

The Latvian Academy of Sciences' Institute of Organic Synthesis has used the solid-phase method to produce a number of short peptides—fragments of the SEB segment homologous to that of thymopoietin II. Some of the fragments have a suppressive effect on lymphoid cells, activate the peritoneal macrophages of mice, and intensify the nonspecific resistance of animals to bacterial infection.

CONCLUSION

Staphylococci can be the cause or an additional adverse factor in various diseases and disorders, from cosmetic defects to life-threatening conditions. Staphylococcal enterotoxins show several types of biological activity, their immunological activity having been studied intensely in recent years. It has been established that the entire enterotoxin molecule is not needed for the manifestation of a given activity, be it antigenic, mitogenic, immunosuppressive, or enterotoxic; but the boundaries of the active centers—the protein chain segments or the surface regions of the globule—have yet to be ascertained. Considering the similarity of the segments of enterotoxin B and of the thymus hormone thymopoietin II, one can assume that one of the immunologically active centers is located in the C-terminal region of the enterotoxin B molecule. In some immunological tests, the comparatively little-studied enterotoxin A shows a high level of activity. The explanation of the molecular mechanisms of action of staphylococcal enterotoxins and the creation on their basis of useful biologically active compounds is a difficult but absorbing problem.

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UDC 612.017:616.155.32-006.446-097

Monoclonal Antibodies as Highly Specific Diagnostic Reagents for Hemopoietic and Lymphoid Neoplasms

18400472 Kiev VISNYK AKADEMIYI NAUK UKRAYINSKOYI RSR in Ukrainian No 6, Jun 88 pp 33-37

[Article by V. H. Pinchuk, corresponding member, UkSSR Academy of Sciences, D. F. Hluzman, doctor of medical sciences, S. P. Sydorenko, candidate of biological sciences, and O. P. Vyetrova, candidate of medical sciences]

[Abstract] A brief review is presented of hybridoma technology used to generate monoclonal antibodies, with description of two such antibodies used as specific reagents suitable for identification of B cells. The antibodies, designated IPO-3 and IPO-10, were tested for reactivity with a series of different B and T lines and shown to react exclusively with the former. Analysis of the published literature demonstrated that IPO-3 and IPO-10 react with previously unrecognized antigenic determinants on B cells from normal and lymphoproliferative conditions, and may be used in immunocytochemical diagnosis of non-Hodgkins lymphomas, lymphogranulamatoses, and differentiation of chronic lympholeukemias. IPO-3 was found to be specific for B-cell blast forms at antigen-dependent stages of differentiation, while IPO-10 detected an antigenic determinant present at various stages of B cell maturation but not on immature B cells in the bone marrow, prior to the appearance of plasma cell precursors. Both sets of monoclonal antibodies, IPO-3 and IPO-10, were obtained in the Laboratory of Cytochemistry and Immunocytology, Institute of Oncological Problems, Ukrainian SSR Academy of Sciences, and made available to other Soviet and COMECON research institutions. Figures 1; tables 2; references 16: 7 Russian, 9 Western.

Induction of DNA Strand Cleavage by Visible Laser Light

18400475b Moscow BIOFIZIKA in Russian
Vol 33 No 2, Mar-Apr 88 (manuscript received 24 May 85; in final form 18 Sep 86) pp 242-246

[Article by V. K. Burilkov, Ye. V. Kovarskiy and G. M. Krochik, Institutes of Ecological Genetics and of Applied Physics, Moldavian SSR Academy of Sciences, Kishinev]

[Abstract] Theoretical and experimental studies were conducted on the cleavage of DNA by visible laser light with the intention that such data may provide a basis for regulation of genetic recombination in plants. The specific studies involved DNA derived from lambda phage and complexes with the intercalating agent acridine orange. The complexes were analyzed in terms of the effects of 30 psec pulses of the second harmonic of YAG:Nd light (532 nm). The theoretical calculations demonstrated that double-strand breaks in phage lambda DNA have threshold parameters of 230 impulses with intensities of 3 x 10^9 W/cm^2, corresponding to a 20.7 J/cm^2 dose. Experimental studies with a 50 J/cm^2 dose and intensities of 2.3 to 8.1 GW/cm^2 showed that the yield of high molecular weight fragments decreased while that of lower molecular weight fragments increased. These observations confirmed theoretical calculations to the effect that with an increase in laser intensity the probability of breaks increases, and confirmed the multiphoton mechanism of laser interaction with the DNA-acridine orange complex. Furthermore, the mechanism of action responsible for the double strand breaks has been shown to be different from the mechanism responsible for the "photodynamic effect," involving as it does single photon absorption by the dye and subsequent reaction with oxygen. Figures 2; references 11: 9 Russian, 2 Western.

UDC 591.185.5

Auditory Frequency Discrimination in Dolphins

18400466e Moscow DOKLADY AKADEMII NAUK SSSR in Russian Vol 300 No 4, Jun 88 (manuscript received 1 Dec 87) pp 1013-1016

[Article by A. Ya. Supin and V. V. Popov, Institute of Evolutionary Morphology and Ecology of Animals imeni A. N. Severtsov, USSR Academy of Sciences, Moscow]

[Abstract] In order to obtain a better appreciation of the auditory system of dolphins and their means of communication, electrophysiological studies were employed to assess frequency discrimination in this species of mammals. The experimental studies were conducted on two captive Tursiops truncatus dolphins, using noninvasive electrode pickups to monitor auditory evoked potentials. The acoustic signals consisted of a continuous stimulus generating 1 Pa pressure, with the spectrum limited to a 1.5 octave band. The lower limit of the spectral band varied from 8 to 128 kHz. The electrophysiological responses demonstrated that the auditory system of dolphins is capable of a much finer analysis of low frequency acoustic signals than that of humans. At low frequencies the absolute frequency discrimination in dolphins was in the vicinity of 0.4 to 0.5 kHz^{-1}, significantly lower than the figures obtained for man (20-25 kHz^{-1}). At higher frequencies the relative discrimination values for dolphin and man were, respectively, 30 and 8-13 kHz^{-1}. Figures 3; references 8: 6 Russian, 2 Western.

UDC 577.21

Use of Retroviral Vectors for Expression of Intron-Bearing Chromosomal Genes: Preparation of Biologically Active Tumor Necrosis Factor (Human Beta-Lymphotoxin)

18400468b Moscow DOKLADY AKADEMII NAUK SSSR in Russian Vol 300 No 6, Jun 88 (manuscript received 15 Dec 87) pp 1498-1502

[Article by V. S. Prasolov, R. L. Turetskaya, S. A. Nedospasov, P. M. Chumakov and A. N. Shakhov, Institute of Molecular Biology, USSR Academy of Sciences, Moscow]

[Abstract] A retroviral vector system was designed for the expression of the human β-lymphotoxin gene in rat fibroblasts, as part of a search for an efficient system for expression of intron-containing eukaryotic chromosomal genes. The gene in question is represented by a 2000 base-pair sequence containing three introns. The standard techniques involved a BamHI-EcoRI fragment of the lymphotoxin gene (1433 bases), containing portions of exons I and IV and complete exons II and III, as well as the three introns. The gene fragment was inserted into the EcoRI site of the DNA of retrovirus vector pPS-neo. A two-stage cloning process involved the conversion of the lymphotoxin DNA fragment into an RNA sequence of the vector used to infect the fibroblast culture. Following the release of the vector RNA into the cytoplasm of the fibroblasts and the action of the viral revertase, a cDNA was synthesized containing the lymphotoxin gene. Following integration into the fibroblast genome, individual clones were obtained that synthesized up to 1000 U/ml human β-lymphotoxin. The pPS-neo system was thus demonstrated to be an efficient vector for ensuring expression of genes in cultured eukaryotic cells. Figures 3; references 13: 2 Russian, 11 Western.

UDC 579.6(579.841.11+579.841.24):615.9

Limited Periplasmatic Proteolysis of Diphtheria Toxoid in Escherichia coli and Erwinia carotovora

18400468c Moscow DOKLADY AKADEMII NAUK SSSR in Russian Vol 300 No 6, Jun 88 (manuscript received 15 Jan 88) pp 1503-1505

[Article by A. G. Zdanovskiy, M. V. Zdanovskaya, N. K. Yankovskiy and V. G. Debabov, All-Union Scientific Research Institute of Genetics and Selection of Industrial Microorganisms, Moscow]

[Abstract] An analysis was conducted on the nature of limited proteolysis of diphtheria toxin (DT) in the periplasmatic space of Escherichia coli and Erwinia carotovora, using recombinant plasmids pAB2 and pAB7 bearing a gene for the N-terminal end of DT. Immunoblotting techniques demonstrated that in both cells the periplasmatic space contained a number of DT peptides, the largest of which had a MW of 40,700, rather than the expected DT molecule with a MW exceeding 61,000. In addition, studies with modified pAB2 and pAB7 plasmids, coding for a toxoid lacking the signal peptide, revealed the cytoplasmic presence of toxids with MW greater than 50,000 (absence of the signal peptide precludes secretion into the periplasmatic space). Through the use of additional plasmids coding for different lengths of the C-end of DT, two cleavage sites that are attacked in the periplasmatic space were identified. The first site of cleavage is located between the C-terminus of the B-fragment of DT and the binding site to eukaryotic cell receptors. The second site is located in the region between the hydrophilic N-terminus of the B-fragment and the hydrophobic segment showing structural resemblance to membrane-intercalating proteins. Figures 2; references 10 (Russian).

Rapid Search Algorithm for Nucleotide Sequence Homology

18400475a Moscow BIOFIZIKA in Russian Vol 33 No 2, Mar-Apr 88 (manuscript received 9 Jun 86) pp 229-232

[Article by A. A. Mironov and N. N. Aleksandrov, All-Union Scientific Research Institute for the Genetics and Selection of Industrial Microorganisms, Moscow]

[Abstract] A method is presented of a novel approach for rapid definition and identification of nucleotide sequence homology in DNA, capable of analysis of extended sequences of weak homology due to exchanges, deletions, and block inversions. The theoretical reasoning underlying this approach is based on the fact that similar sequences have a similar nucleotide composition. Consequently, the sequences were assigned vector properties and transformed into matrices with the use of the Monte Carlo modeling method. The number of operations required to compare sequences for a given length of a DNA strand requires only about 10^3 sec of computer time versus ca. 10^5 sec by more conventional methods. The time saving lends itself to the use of the matrix method for quick screening projects. Figures 1; references 3: 1 Russian, 2 Western.

UDC 577.15:572.852.11

Bacillus thuringiensis Restrictase Susceptible to dam-Methylation

18400481a Moscow BIOTEKHNOLOGIYA in Russian Vol 4 No 2, Mar-Apr 88 (manuscript received 30 Nov 87) pp 197-198

[Article by R. R. Azizbekyan, B. A. Rebentish and Ye. M. Netyksa, All-Union Scientific Research Institute of Genetics and Selection of Industrial Microorganisms, Moscow]

[Abstract] Bacillus thuringiensis U44 has been shown to be resistant to a wide spectrum of phages, leading to a study of this strain to define its restrictase system. A restriction endonuclease was identified and tested on the

DNA of a variety of phages, leading to the conclusion that it acted on the ATCGAT site, in analogy to the well-defined Cla I restriction enzyme. Further studies on the cleavage of phage lambda DNA by both enzymes led to identical results showing that both the B. thuringiensis U44 enzyme (designated Btu I) and Cla I are isoschizomers susceptible to dam-methylation. These findings demonstrated that B. thuringiensis possesses two restriction systems for modifying DNA, Btu I and the previously described Bti I, that determine phage susceptibility and horizontal transfer of genetic information. Figures 1; references 6: 2 Russian, 4 Western.

Frequency Range of UHF Auditory Effects

18400475e Moscow BIOFIZIKA in Russian
Vol 33 No 2, Mar-Apr 88 (manuscript received 5 Aug 86) pp 349-350

[Article by R. E. Tigranyan and V. V. Shorokhov, Institute of Biological Physics, USSR Academy of Sciences, Pushchino, Moscow Oblast]

[Abstract] Previous studies have indicated that the lower limit of human perception of radiosignals is represented by 8 kHz, while studies on physical models suggested that humans should be able to perceive the entire sound range. Consequently, additional studies were undertaken on three subjects that, for the first time, gave evidence of perception of zero beats of a radiosignal from an electrodynamic emitter below 8 kHz. The subjects were exposed to beats in the preresonance band (1-7 kHz, 0.6 W/cm^2) at low noise levels (ca. 20 dB). Duration of the UHF impulses was limited to a maximum of 25 microseconds. Beat perception was most acute at the following frequencies: 3.58, 4.21, 5.23, and 6.99 kHz for the first subject, 4.01, 5.33, and 6.99 kHz for the second, and at 3.80, 4.74, and 4.97 kHz for the third. Only one of the subjects perceived a sound in the 1-3 kHz range, with all subjects showing increased perception of zero beats with tonal acoustic signals beginning at 3 kHz. References 4: 2 Russian, 2 Western.

UDC 577.391;577.44;323.4

Delayed Behavioral Activation After Single Microwave Exposure

18400479h Moscow RADIOBIOLOGIYA in Russian
Vol 28 No 2, Mar-Apr 88 (manuscript received 29 Apr 87) pp 281-283

[Article by M. A. Navakatikyan and S. I. Nogachevskaya, Kiev Scientific Research Institute of General and Communal Hygiene imeni A. N. Marzeyev, Ukrainian SSR Ministry of Health, Kiev]

[Abstract] A study was conducted on the behavioral sequelae of a single exposure to microwave irradiation (2450 MHz, 1 mW/cm^2, 0.27 mW/g for 7 h) of Fisher-344 female rats, to further define longer term effects of microwaves. Analysis of maze behavior and evaluation of individual forms of motor activity demonstrated that behavior was not affected within the time span of a day after exposure. However, behavioral activity was enhanced after 4 days. Delayed activation of the CNS was interpreted to indicate that the effects of the microwaves went beyond cutaneous receptors and eventually involved the hypothalamus-pituitary-peripheral endocrine gland axis. Tables 1; references 8: 4 Russian, 4 Western.

Enkephalin Analog Design at the Institute of Organic Synthesis of the Latvian Academy of Sciences

18400461 Riga IZVESTIYA AKADEMII NAUK LATVIYSKOY SSR in Russian No 5, May 88 pp 64-71

[Article by I. V. Bobrova, Yu. Yu. Balodis, G. V. Nikiforovich, and G. I. Chipens, Red Banner of Labor Institute of Organic Synthesis, Latvian SSR Academy of Sciences]

[Text] The basic principles of the design of analogs of natural bioregulators were established at the Institute of Organic Synthesis of the Latvian Academy of Sciences several years before the discovery of enkephalins. Thus, as early as 1970, researchers[1] had developed the concept that the biological action of any pharmacological agent is determined by its signature—the set of properties that determine all the stages of interaction between an agent and a receptor, from primary identification to the ligand-receptor generation of the secondary, intracellular signal that initiates the biological reaction. In 1973 researchers[2] proposed that the pharmacological effects of many empirically identified medicinal preparations are due to the similarity of their signatures to those of natural bioregulators—substances of endogenic origin that are capable of performing the very same biological functions, albeit, possibly, more successfully, because the mutual "adaptation" of such endogenic substances and their corresponding receptors is made over the course of long-term mutual evolution. When, in 1975, the first reports of the discovery of enkephalins and endorphins appeared[3], it became immediately apparent that, at least in relation to interaction with opiate receptors, enkephalins actually have signatures similar to that of morphine.

On the other hand, in addition to its strong analgesic action, morphine itself has a whole series of undesirable side effects: suppression of respiration, reduced blood pressure, addiction, tolerance, and withdrawal. This prompted a rather extensive search for morphine analogs that would be more suitable as medicinal preparations than morphine itself. At present, several dozens of analogs are known, and they can be categorized according to two parameters: α, the magnitude of "internal" activity; and pD_2 (the negative logarithm of the dissociation constant of the ligand-receptor complex), which was introduced into pharmacology by Ariens[4]. The parameters of some of these compounds are presented in Figure 1 on a coordinate system on whose axes are plotted the characteristics of the internal activity and similarity. The figure depicts, specifically, buprenorphine, which is considered one of the best morphine-like preparations: although a strong analgesic, it does not lead to addiction or physical dependence and is a partial antagonist of the narcotic action of morphine[5]. Moreover, buprenorphine has an extremely long period of action, which indicates the formation of extremely stable complexes with opiate receptors, as Figure 1 shows. One can assume that, with buprenorphine, the slow breakdown itself of the ligand-receptor complex ensures the absence of withdrawal, which, in the opinion of the authors of the work reported previously[6], is associated with the inhibited release of adenylate cyclase and, correspondingly, with the slow restoration of the intracellular level of cyclic AMP. Thus, the example of buprenorphine shows that the best medicinal preparations, in terms of preserving the signature of morphine, are apparently those that (1) form the sturdiest ligand-receptor complex, ensuring its slow dissociation, and that (2) activate the cell-receptor system comparatively mildly (have an average α coefficient). The discourses above cannot, of course, be considered specific recommendations: they are capable only of indicating the general direction taken by the design of morphine-like preparations, including a number of enkephalins. At this point, certain distinctions between the manifestations of the biological effects of morphine analogs and enkephalins must be mentioned. First, for enkephalins more so than for morphine analogs, a heterogeneity of receptors is expressed, i.e., the existence of receptor types such as μ, δ, and κ[7]. Second, the study of enkephalins has shown that analgesia is only part of their extremely broad spectrum of biological action. They affect, for example, the cardiovascular system, gastrointestinal tract functions, temperature regulation, and behavioral responses.

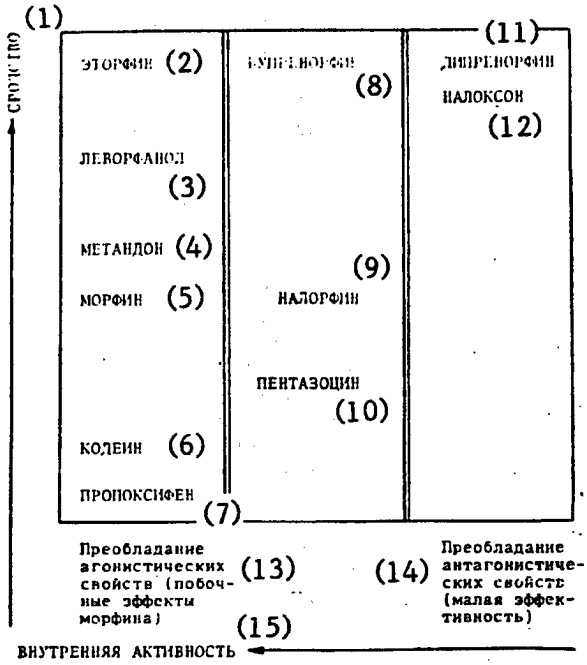


Figure 1. Schematic representation of the balance of properties of morphine analogs when they interact with cell receptors.

Key:—1. Similarity—2. Etorphine—3. Levorphanol—4. Methadone—5. Morphine—6. Codeine—7. Propoxyphene—8. Buprenorphine—9. Nalorphine—10. Pentazocine—11. Diprenorphine—12. Naloxone—13. Predominance of agonistic properties (side effects of morphine)—14. Predominance of antagonistic properties (low effectiveness)—15. Internal activity

Enkephalins and other opioid peptides stimulate the release of prolactin, growth hormone, corticotropin, and vasopressin and lower the secretion of gonadotropin and thyrotropin. The distribution of opioid peptides in the central nervous system (CNS) indicates that enkephalins are capable of acting as short-term neuromediators and that endorphins are capable of acting as long-term neuromediators.

Nevertheless, the common formulated problem of design—the search for compounds that are highly similar to receptors—remains pressing for enkephalin analogs. What is more, upon deriving analogs that are more similar to various types of receptors, one can look forward to effecting a selectivity of action of these analogs, which, as can be seen from the paragraph above, is of especial significance for enkephalins. Considering also the extremely high lability of peptides in relation to enzymatic cleavage, one would hope that the resultant enkephalin analogs would be resistant to proteases and would make it possible, for example, to perform intravenous injection.

Generally speaking, a wide variety of factors influence the effectiveness of the biological action of analogs of peptide bioregulators (a detailed survey has been presented in a previous monograph[8]). Specifically, more than anything else the presence of certain functional groups emerging as bearers of the signature of the natural compound affects the α value of an analog. In the opinion of most researchers, aromatic nuclei of side chains of Tyr and Phe residues are just such groups for the enkephalin: they, above all (with the presence of the α-amino group), represent the elements of similarity between the morphine and enkephalin molecules. However, the pD_2 value, i.e., the degree of similarity to the receptors, is determined almost exclusively by molecule conformation, or more precisely, by how similar the peptide conformation in "biophase" near the receptor surface is to the so-called biologically active conformation—the structure that the peptide acquires in direct interaction with the receptor. Thus, it becomes possible to more accurately determine the target of a focused search for enkephalin analogs: analogs whose conformation is as close to the biologically active structure as possible must be sought. Serving as an example of such analogs are cyclic analogs with stabilized biologically active conformations, which raises its statistical weight among other molecule conformers and ensures a higher degree of recognition of the peptide by a specific receptor and, as a result, a tighter bond with it. The LaSSR Academy of Sciences Institute of Organic Synthesis has garnered specific experience in creating similar analogs of tuftsin[9] and bradykinin[10], managing in the latter instance to achieve pronounced selectivity of cyclic analogs and prolongation of their action—apparently, the result of increasing their resistance to enzymatic cleavage.

This method of the molecular design of enkephalin analogs requires, naturally, as detailed a representation as possible of the biologically active structure of the natural compound. Such a representation, in turn, can be produced with semiempirical conformation analysis: serving as the biologically active conformation is the peptide core structure common to sets of low-energy structures of the natural peptide and its analogs that have a high degree of similarity to receptors and absent from the set of low-energy structures of analogs that have a low degree of similarity to receptors. This principle, which we applied to an enkephalin molecule for the first time ever[11], made it possible to propose a model of the biologically active conformation of a molecule, which is depicted in Figure 2. We chose that structure as our starting model. The term "starting model" is not accidental: the fact is that the model in Figure 2 was proposed without regard for a series of important factors that affect enkephalin activity; specifically, without regard for the heterogeneity of opiate receptors. Therefore, in the course of controlled synthesis of enkephalin analogs, such a model must invariably be refined; one possible process for refining the model in presented in Figure 3. It is apparent on the diagram that a fundamental role in the refinement of the model is played by the synthesis and biological testing of conformationally restricted enkephalin analogs and by their semiempirical conformation analysis, with the help of which one can, to some extent, model the conditions enveloping a molecule when planting on a receptor biophase less polar than an aqueous medium (a more detailed account has been given previously[12]). In that context, physicochemical methods of studying spatial structure—Raman, IR, and NMR spectroscopy—are, generally speaking, less informative, because their results depend substantially on the type of solvent in which the molecule is located and, besides, are ambiguous. The results of x-ray structural analysis are less ambiguous, although the most reliable of the enkephalin structures derived in this manner is a completely elongated conformation known to have nothing in common with the biologically active structure[13]. At the same time, biological testing methods can yield much in the solution of the problem of refining the biologically active enkephalin conformation. However, not all of them are equivalent in this regard. Thus, analgesic peptide activity, primarily, is studied when

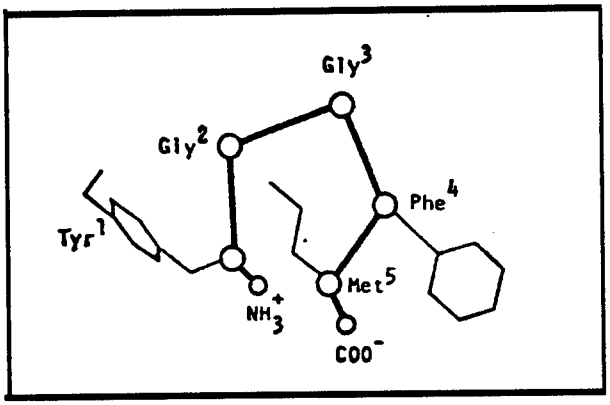


Figure 2. Starting model of the biologically active enkephalin conformation.

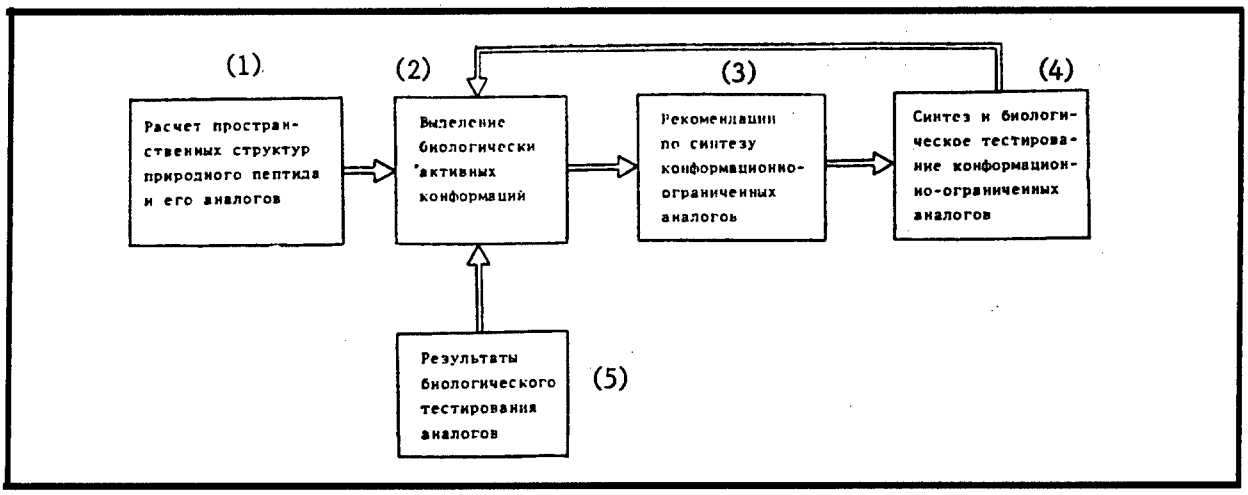


Figure 3. Schematic of directed search for effective analogs of peptide bioregulators based on the conformational properties of their molecules.

Key:—1. Calculation of the three-dimensional structures of the natural peptide and its analogs—2. Selection of the biologically active conformations—3. Recommendations on the synthesis of conformationally restricted analogs—4. Synthesis and biological testing of conformationally restricted analogs—5. Results of biological testing of analogs

morphine-like activity in vivo is being evaluated. An analog is introduced into an animal with a given method—into the lateral ventricle of the brain, intravenously, subcutaneously, or perorally—and the analgesic effect is evaluated on the basis of the extent of the delay of the standard reaction of the animal—tail jerk, tail pinch, and hot plate tests. The very same methods, for the most part, are also used for the study of classical opiates. It is obvious that, although these tests are more interesting from the standpoint of creating a practically useful preparation (this especially relates to intravenous injection), they are poorly informative for studying the mechanism of action of an enkephalin—if only because, with the heterogeneity of opiate receptors, it is not known through which type of receptor the analgesic effect is mediated in vivo. Moreover, because of the diversity of the methodological approaches, the in vivo enkephalin activity data obtained in various laboratories do not always coincide.

More closely modeling the situation arising in the interaction of opiates and enkephalin analogs with various types of receptors are isolated organ (in vitro) tests based on the ability of opiates to inhibit electrically stimulated contractions of the ileum of a guinea pig and the vas deferens of a mouse. A number of studies have shown that μ-type receptors are typical for the first of those preparations, and δ-type receptors, for the second[14, 15]. However, the most reliable in terms of the direct study of the binding of enkephalin analogs with certain types of receptors is, obviously, radioreceptor analysis that is based on the determination of the ability of the substance under study to vie for a stereospecific binding site on synaptic membranes of the brain and that uses a radioactively labelled ligand selective for a given subclass of opiate receptor.

In the process described below, which involved the controlled design of conformationally restricted enkephalin analogs, we used all the biological testing methods described above, with an apparatus that used semiempirical conformation analysis for the study of the spatial structure of synthesized enkephalin analogs. The testing was performed by Candidate of Biological Sciences N. A. Abissova, under the direction of Doctor of Medical Sciences V. Ye. Klushi and Candidate of Chemical Sciences G. F. Rozental.

The biologically active conformation of the enkephalin molecule, depicted in Figure 2, has two basic features: the convergence of the ends of the molecule, with the formation of a likeness of the β-loop in the region of residues 2-3, and the spatial equivalence of side chains in positions 2 and 5. In addition, it is important that the aromatic nuclei of the Tyr and Phe residues are located "on different sides" of the molecule. Once all these circumstances are taken into consideration, one can immediately suggest several types of analogs that would make it possible to secure (or imitate) the potential biologically active conformation of Figure 2 (schematically depicted in Figure 4, structural type 1). To begin with, the joining of the N- and C-ends of the molecule seems perfectly natural (structure 2 of Figure 4). Furthermore, since, according to the model in Figure 2, there is a definite equivalence in the spatial arrangement of the side chains of the molecule in positions 2 and 5, one can assume that the presence of a side chain in position 2 will not substantially alter the biologically active conformation (structure 3, Figure 4). Stabilizing the structure with the union of positions 2 and 5 with a valence bond (structure 4) also seems perfectly natural. Since a given arrangement of aromatic nuclei is enough, as has been indicated, for reproducing the signature of morphine,

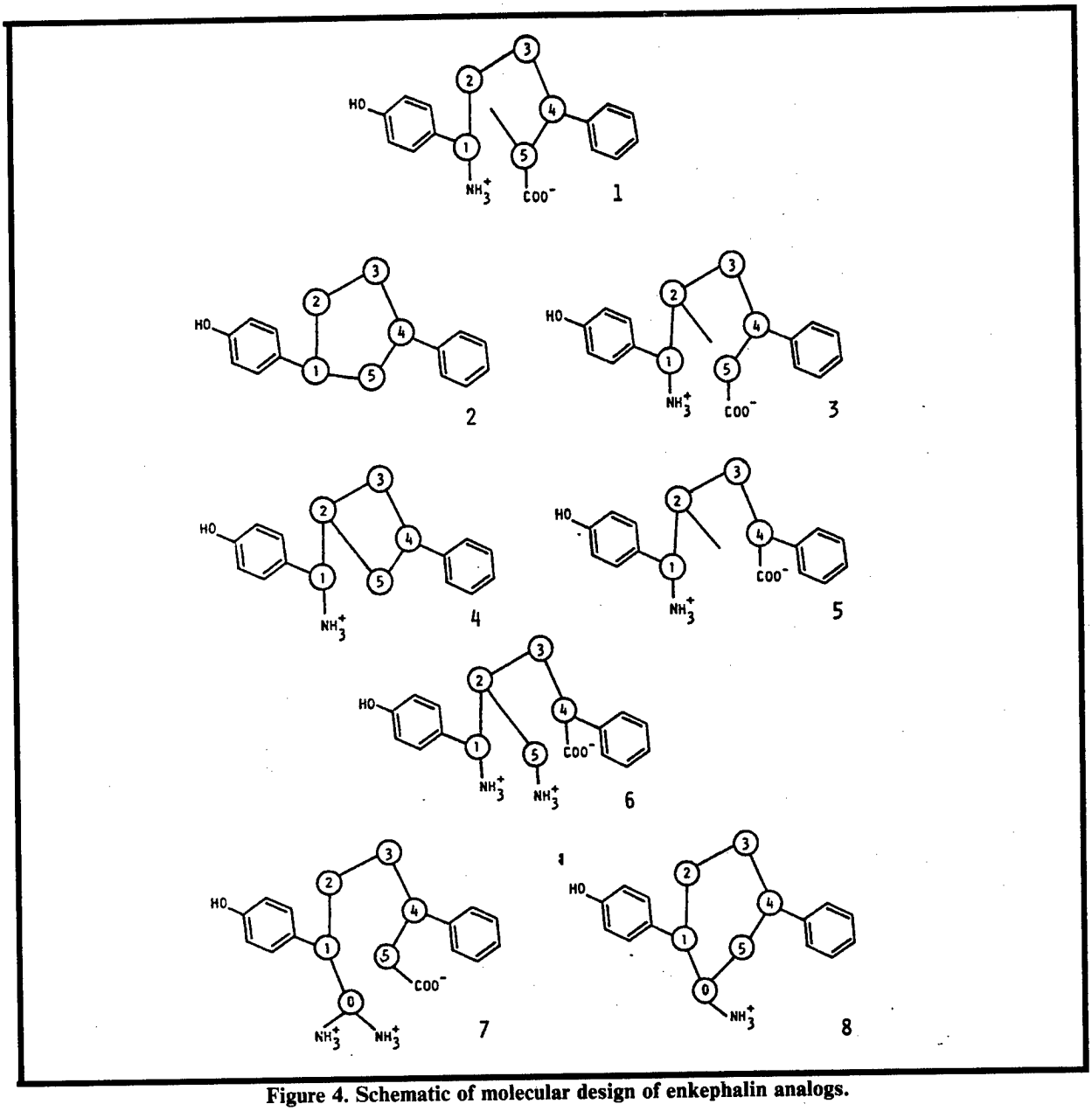
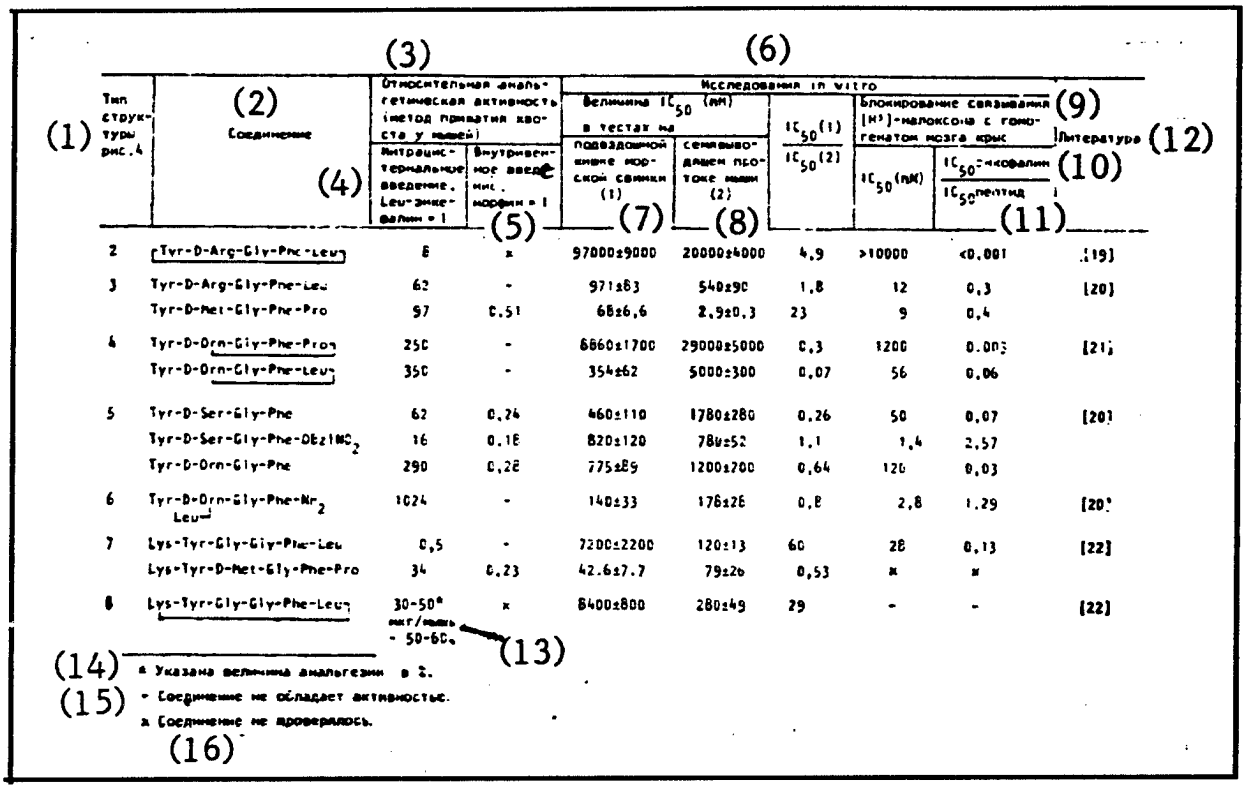


Figure 4. Schematic of molecular design of enkephalin analogs.

one can try to condense the size of the molecule to a tetrapeptide with the transfer of the side chain from position 5 to position 2 (structure 5). The homology of the biologically active conformation can also be preserved by structural type 6 of Figure 4, obtained by breaking the bond between the amino acid residues in positions 4 and 5 of the cyclopeptide of structure 4; in this manner, new, "branched" enkephalin analogs can be formed. One can also assume that the appearance in position 0 of the basic residue with the free amino group (structure 7), and consequently the cyclization of the molecule by means of this residue (structure 8, Figure 4), will facilitate the maintenance of the biologically active conformation as well as protection from premature enzymatic cleavage of the molecule.

We began a focused search for enkephalin analogs of the types shown in Figure 4 in the late 1970s; in the past, the literature had described several hundreds of enkephalin analogs, among which, naturally, were some similar to those we synthesized. Thus, our findings, summarized in the table, may be compared with the data of other researchers[8]. So, for example, type 2 compounds that were synthesized by other authors did not, as a rule, manifest any kinds of indices of biological activity. Against that background, a cyclic analog created by us



(1) Тип структуры рис.4	(2) Соединение	(3) Относительная анальгетическая активность (метод прижатия хвоста у мышей)		(6) Исследования in vitro					
		(4) Интрацистернальное введение. Leu-энкефалин = 1	(5) Внутривенное введение. морфин = 1	Величина IC_{50} (нМ) в тестах на (7) подвздошной кишке морской свинки (1)	(8) семявыносящем протоке мыши (2)	$IC_{50}(1)/IC_{50}(2)$	(9) Блокирование связывания [H^3]-налоксона с гомогенатом мозга крыс IC_{50} (нМ)	(10) IC_{50} энкефалин / (11) IC_{50} пептид	(12) Литература
2	Tyr-D-Arg-Gly-Phe-Leu	8	x	97000±9000	20000±4000	4,9	>10000	<0.001	[19]
3	Tyr-D-Arg-Gly-Phe-Leu	62	-	971±83	540±90	1,8	12	0,3	[20]
	Tyr-D-Met-Gly-Phe-Pro	97	0,51	68±6,6	2,9±0,3	23	9	0,4	
4	Tyr-D-Orn-Gly-Phe-Pro	250	-	6860±1700	29000±5000	0,3	1200	0.003	[21]
	Tyr-D-Orn-Gly-Phe-Leu	350	-	354±62	5000±300	0,07	56	0,06	
5	Tyr-D-Ser-Gly-Phe	62	0,24	460±110	1780±280	0,26	50	0,07	[20]
	Tyr-D-Ser-Gly-Phe-OBzlNO$_2$	16	0,18	820±120	780±52	1,1	1,4	2,57	
	Tyr-D-Orn-Gly-Phe	290	0,28	775±89	1200±200	0,64	120	0,03	
6	Tyr-D-Orn-Gly-Phe-Nr$_2$ Leu	1024	-	140±33	176±26	0,8	2,8	1.29	[20]
7	Lys-Tyr-Gly-Gly-Phe-Leu	0,5	-	7200±2200	120±13	60	28	0,13	[22]
	Lys-Tyr-D-Met-Gly-Phe-Pro	34	0,23	42.6±7.7	79±26	0,53	x	x	
8	Lys-Tyr-Gly-Gly-Phe-Leu	30-50* мкг/мышь - 50-60 (13)	x	8400±800	280±49	29	-	-	[22]

(14) * Указана величина анальгезии в %.
(15) - Соединение не обладает активностью.
x Соединение не проверялось.
(16)

Key:—1. Type of structure, Fig. 4—2. Compound—3. Relative analgesic activity (tail pinch method in mice)—4. Intracisternal injection, Leu-enkephalin = 1—5. Intravenous injection, morphine = 1—6. In vitro study—7. IC_{50} (nM) in tests on guinea pig ileum (1)—8. IC_{50} (nM) in tests on mouse vas deferens (2)—9. Blockade of binding of [H^3]-naloxone with rat brain homogenate—10. IC_{50} enkephalin—11. IC_{50} peptide—12. Reference no.—13. μg/mouse—14. *Analgesia level indicated in percent—15. - Compound shows no activity—16. x Compound not tested

would seem more promising, since it has some activity upon intracerebral injection. Upon in vitro study, however, it turned out to be ineffective. Type 3 compounds have also been studied by other authors: one of the most actively studied among them is the Tyr-D-Met-Gly-Phe-Pro analog, examined by us for the first time ever in a previous study[6], which showed that its conformation corresponded to the suggested biologically active conformation. Of interest is the Tyr-D-Arg-Gly-Phe-Leu analog synthesized by us and subjected to a painstaking conformational analysis in a previous study[17]. The type 4 compounds we synthesized are comparable in terms of the level of biological effects they induce to analogs of the same type produced by other authors; conformational analysis of similar compounds made it possible to perform the first correction of the model of the biologically active conformation of the enkephalin molecule[8]. That correction addresses only the specific biologically active conformation of fragments 1-4, whereas the arrangement of the residue in position 5 is not so important: in general terms, the refined biologically active conformation is distinguished from the structure in Figure 2 only by some type of β-like bend of the core. The correction made of the model is confirmed by the fact that the type 5 synthesized compounds, which are tetrapeptides, show considerable activity upon intravenous injection. Type 6 analogs, unlike all those enumerated above, are completely new; among them, as can be seen, are compounds with pronounced analgesic activity. The rather widely used type 7 compounds are, essentially, merely starter compounds for producing new, type 8 analogs: unfortunately, the example of this analog listed in the table is of little interest, because the analog shows only weak activity upon intracerebral injection. In terms of conformation, as the calculations show, the analog is rather mobile and has, among many other low-energy structures, a conformation of the "refined" biologically active type[18].

Summing up the survey of data on the biological testing of the analogs that we synthesized and that are listed in the table, we note that no correlation is observed between the level of analgesic activity of the analogs and the values that characterize their similarity to a given opiate receptor—either μ or δ. This circumstance, first, does not yet enable us to divide the model of biologically active enkephalin conformations into types of structures typical of a given type of receptor; and, second, it prompts us to express some doubt about the correctness of the currently prevalent concept that the analgesic

activity of enkephalins (and, in general, of the ligands of opiate receptors) is mediated exclusively through μ receptors.

In conclusion, we can state that, as can be seen, the approaches we developed to the molecular design of enkephalin analogs on the whole are quite effective: we found a whole series of analogs that are more active than enkephalin upon intracerebral injection, and some of them are comparable to morphine upon intravenous injection.

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UDC 615.214:547.245.246'582.5

Neurotropic Effects of Beta-Silyl, -Germyl, and -Stannyl Derivatives of Hydroxamic Acids

18400473a Riga IZVESTIYA AKADEMII NAUK LATVIYSKOY SSR in Russian No 5, May 88 (manuscript received 7 Feb 88) pp 79-82

[Article by E. Ya. Lukevits, S. K. Germane, M. A. Trushule, A. Ye. Feoktistov and V. F. Mironov, Order of the Red Banner of Labor Institute of Organic Synthesis, Latvian SSR Academy of Sciences; Order of the Red Banner of Labor State Scientific Research Institute of the Chemistry and Technology of Heteroorganic Compounds]

[Abstract] A comparative pharmacodynamic and toxicological study was conducted on β-silyl-, -germyl-, and -stannyl-substituted propio- and isobutyrohydroxamic acids to assess their potential application as CNS agents. The studies conducted on outbred mice showed that on intraperitoneal administration the LD_{50} for the Sn congener of propiohydroxamic acid (20.5 mg/kg) was 100-fold greater than for the Ge congener (2000 mg/kg), and 40-fold greater than for the Si congener (815 mg/kg). β-Trimethylgermylpropiohydroxamic acid was found to be the most effective antihypoxic agent, increasing the survival time of mice subjected to hypoxic hypoxia by 206.6% in a dose of 5 mg/kg. The isobutyrohydroxamic acid compounds showed toxicity patterns similar to the propiohydroxamic acid compounds, but with less spread among the various congeners. None of the compounds protected the experimental animals against convulsions induced by electric current but mitigated the convulsive consequences of Corazole administration. In the latter case, the trimethylstannyl derivatives of both propio- and isobutyrohydroxamic acids were most effective, increasing the lethal dose of Corazole 3.26- and 2.14-fold, respectively. Tables 2; references 8 (Russian).

UDC 547.967.4:612.81

Effects of Nerve Growth Factor Fragment on Learning and Memory as Modulated by Magnetic Fields

18400473b Riga IZVESTIYA AKADEMII NAUK LATVIYSKOY SSR in Russian No 5, May 88 (manuscript received 22 May 87) pp 83-86

[Article by A. A. Folomkina, R. A. Danilova, Ch. A. Asabayev, G. I. Chipens, O. S. Papsuyevich, Yu. A. Kholodov, R. I. Kruglikov and S. S. Sadretdinov, Institute of Higher Nervous Activity and Neurophysiology, USSR Academy of Sciences; Moscow State University imeni M. V. Lomonosov; Order of the Red Banner of Labor Institute of Organic Synthesis, Latvian SSR Academy of Sciences; Tashkent Pharmaceutical Institute, Uzbek SSR Ministry of Health]

[Abstract] The role of the 48-59 amino acid fragment of the nerve growth factor in learning and memory was investigated on model systems provided by 130-180 g outbred rats, using selected conditioned reflexes and subcutaneous administration of the peptide in a 5 μg dose. Administration of the peptide had a deleterious effect on the development and retention of passive avoidance reflexes, whereas it was rather innocuous with respect to the development of conditioned dual avoidance reflexes. The effects of the peptide on active avoidance conditioned reflexes were predominantly of an amnesic nature. The combined effects of the peptide and of concomtitant exposure to an alternating sinusoidal magnetic field (3 mT, 10 Hz) intensified the amnesia two-fold. Since the peptide was administered immediately after a training session its physiological mechanism of action evidently affected consolidation of memory engrams. Tables 3; references 13: 3 Russian, 10 Western.

UDC 547.495.9:615.277.3

Release Kinetics of Putative Antineoplastic Agent Polyhexamethyleneguanidine Phosphate From Magnetic Microspheres

18400473c Riga IZVESTIYA AKADEMII NAUK LATVIYSKOY SSR in Russian No 5, May 88 (manuscript received 30 Jun 87) pp 87-91

[Article by L. E. Markevicha, R. A. Paegle, E. Ya. Blum and M. Yu. Lidak, Institute of Physics and the Order of the Red Banner of Labor Institute of Organic Synthesis, Latvian SSR Academy of Sciences]

[Abstract] Studies were conducted on the release kinetics of polyhexamethyleneguanidine phosphate (PMGP) from magnetic microspheres prepared by Widder's method [Widder, K., et al., J. Pharm. Sci., 68(1): 79, 1979]. Magnetic properties were imparted to the microspheres by 10 nm magnetite particles. The microspheres ranged in size up to a diameter of 10 μm and were prepared from PMGP, magnetite, and albumin in ratios of 1:2:20, 0.75:2:20, and 0.5:2:20. Solidification was

conducted in cottonseed oil at 120, 140, and 160°C. Evaluation of the release kinetics of PMGP, a putative antineoplastic agent, was dependent on temperature and size of the microspheres. The release at 22°C after 30 min in distilled water was some 15% less than at 40°C, with 75% of the PMGP released at 40°C vs. 58% at 22°C. In addition, the rate of release was inversely proportional to the average diameter of the microspheres. The data indicated that PMGP is released from the microspheres by diffusion as a result of swelling of the albumin. In addition, at higher temperature, e.g., 40°C, partial solubilization of albumin also favors loss of PMGP from the microspheres. Figures 5; tables 1; references 5: 1 Russian, 4 Western.

UDC 591.089.84.612.8

Recovery of Normal RNA Synthesis in Rat Cortex After Hypoxia and Transplantation of Embryonal Nervous Tissue

18400468a Moscow DOKLADY AKADEMII NAUK SSSR in Russian Vol 300 No 6, Jun 88 (manuscript received 25 Jan 88) pp 1477-1481

[Article by L. V. Polezhayev, I. N. Saburina, L. V. Cherkasova, V. N. Vitvitskiy and A. V. Timonin, Institute of General Genetics imeni N. I. Vavilov, USSR Academy of Sciences, Moscow]

[Abstract] The effects of hypoxic hypoxia and embryonal nervous tissue implants on cortical RNA synthesis were studied in male Wistar rats (150-200 g), employing ^{14}C-adenine incorporation and autoradiography. Transplantations were conducted 25 days after a hypoxic episode, consisting of a 5-7 mm^3 transplant implanted into the parietal lobe. The transplants were derived from 19-day old Wistar embryos. Hypoxic hypoxia resulted in depression of RNA synthesis in both the neural and glial components of the cortex. A sharp increase in RNA synthesis was engendered by mechanical trauma. Implants led to recovery of normal patterns of RNA synthesis in the neural and glial cells in both cerebral hemispheres of rats subjected to hypoxia. These observations confirm previous results obtained in analogous studies and suggest that such findings may be of clinical interest. Figures 3; tables 3; references 7: 5 Russian, 2 Western.

UDC 591.089.84:612.8

DNA and Protein Synthesis in Rat Cortex Following Hypoxic Hypoxia and Transplantation of Embryonal Neural Tissue

18400480 Moscow ZHURNAL OBSHCHEY BIOLOGII in Russian Vol 49 No 3, May-Jun 88 (manuscript received 26 May 86) pp 355-364

[Article by V. N. Vitvitskiy, Institute of General Genetics, USSR Academy of Sciences, Moscow]

[Abstract] The effects of a biopsy-needle lesion and implantation of embryonal nervous tissue on DNA and protein synthesis in the cortical tissue of Wistar rats (180-240 g) were assessed in terms of ^{3}H-thymidine and ^{3}H-leucine uptake. The multiple factor experiments, including various controls and hypoxic hypoxia, demonstrated unequivocally that cortical transplants from 17-day old rat embryos enhance DNA and protein synthesis. The effects of the transplanted tissue were evident both at the site of the implant and at distances of 6 mm from the site of the lesion in the immediate (4 days) and distal (100 days) time frames. The increase in the rates of DNA and protein syntheses were due to neurotropic factors produced by the damaged tissue, as well as factors synthesized by the developing embryonal neural tissue. The latter factors were seen to exert a much more profound effect in stimulating DNA and protein synthesis. Designated as ontogenetic factors, the latter were felt to differ significantly from the nerve growth factors and to be synthesized only at specific developmental stages. Figures 4; references 14: 10 Russian, 4 Western.

Changes in Granulo- and Monocytopoiesis and in the Activity of Human Stromal Cell Precursors during Acute Hypobaric Hypoxia

18400512b Leningrad TSITOLOGIYA in Russian Vol 30 No 4, Apr 88 (manuscript received 23 Jan 87) pp 466-470

[Article by L. V. Filev, S. F. Yenokhin, N. N. Kotsyubinskiy, D. I. Korotkov, B. G. Liparteliani, A. A. Chernykh, and A. S. Brezgin, Military Medical Academy, Leningrad]

[Abstract] The rise in morbidity in acute hypobaric hypoxia (AHH) is associated with marked changes in the immune system, i.e., intensified destruction of lymphocytes and a decrease in their activity. Ten healthy male volunteers, 18-24 years old, were subjected to AHH for 48 hrs (equivalent to an altitude of 4,000 m). The "ascent" to the "height" and the "descent" both lasted 30 min. Blood and bone marrow were sampled immediately prior to entering the chamber and after emerging from it. It was shown that for 48 hours the number of granulocytes, lymphocytes and monocytes in the blood did not change; the fraction of parabiotic and nonviable leukocytes increased. Bone marrow cellularity did not change, but the fraction of nonviable myelokaryocytes increased. It was shown that during AHH a change occurs in granulo- and monocytopoiesis at the level of circulating leukocytes, bone marrow cells and precursor cells: leukocyte and myelokaryocyte damage was intensified, bone marrow reserves were depleted and the system of mononuclear phagocytes was activated. These changes could lead to higher morbidity and diminished cicatricial and bone tissue repair processes at high altitudes. References 23: 22 Russian, 1 Western.

Kuzin Discusses Physicians' Lack of Practical Skills

18400477 Moscow NEW TIMES in English No 25, Jun 88 pp 30-32

[Interview by NEW TIMES correspondents with Professor Mikhail Kuzin: "Test for a Sense of Compassion"]

[Text] Can you teach mercy? Is the production-line method applicable to surgery? Is the opinion that a "fashionable doctor" will give better treatment always right? These and other questions by our correspondents were answered by Professor Mikhail Kuzin, a member of the Presidium of the U.S.S.R. Academy of Medical Sciences.

The interview took place when he had already decided to resign from his post of director of the Vishnevsky Institute of Surgery under the U.S.S.R. Academy of Medical Sciences so as to fully dedicate himself to research and teaching work he had been doing at the Burdenko Surgical Clinic of the First Moscow Medical Institute for a quarter of a century. Professor Kuzin is also co-chairman of the Soviet Committee of Physicians for the Prevention of Nuclear War.

New Times. There was much talk in previous years about the advantages of the Soviet public health system, its achievements, our country leading the world in the number of doctors and hospital beds, and the unique surgical operations performed here. At the same time we are, unfortunately, by no means among the world's leaders in life expectancy, and the situation is hardly any better as far as some chronic and occupational diseases are concerned. What is the main contradiction here, in your opinion?

Mikhail Kuzin. Indeed, the achievements of Soviet medicine, particularly in the field of surgery, are universally recognized. Nevertheless, we've failed to stay ahead in a number of fields—owing perhaps to serious faults in the last two links of the demand-supply-financing-realization chain. As for life expectancy, mortality rates and chronic and occupational diseases, these are only partially connected with the state of public health. They depend not so much on the quality of the public health service as on social factors, specifically on each individual's lifestyle: his regimen, diet, addiction to or abstention from smoking and drinking, as well as on the observance of safety rules in production, on whether the economy is managed rationally, and the state of the air, the water and the soil. The health of a nation is directly dependent on its ecology.

Perestroika has pushed into the limelight problems that have accumulated in the public health system for years and are integrated in the social life more than any other. Apart from attaching to them the significance they call for, correct ways must be found to tackle them. Let me explain what I mean.

We do have a large number of hospital beds, but people often have to wait for months for their turn to be operated on. Will the situation improve if we have two or three times as many hospital beds as we do now? I don't think so, because hospitals make extremely inefficient use of their bed space.

Here is an example from the common practice of most hospitals: a widespread illness like varicosis calls as a rule for surgical treatment and a 20-day in-patient stay in hospital. We at the Institute of Surgery have suggested a way of reducing the period of varicosis patients' stay in hospital to four or five days! In an experiment which involved over 1,500 patients we gave varicosis cases out-patient examinations at our institute before their admission to a hospital, which was immediately followed by an operation and, within two or three days of that, by their discharge (unless there were complications, of course). The course ended with a 10- to 12-day period of follow-up checks for which a patient had to report to us or to his district clinic. Skeptics called this practice into question, but the results surpassed all our expectations: our patients' leaves were halved, and the incidence of post-operative complications fell drastically. Naturally, the state too benefits from this.

We have received many letters of gratitude, particularly from mothers of big families for whom it is not easy to be away from home for 20 days. Surprisingly, this method has left even our Moscow colleagues totally cold; no one has responded to our suggestion that at least one clinic should give the idea a "field test." I could give you many more cases in point, although it seems to have become clear to all by now that medicine, like the economy, has to develop in an intensive rather than extensive way...

Although we do have more doctors than any other country in the world, we are somehow always short of them. This is due, among other things, to the fact that too many medical experts who might otherwise have been practicing physicians are employed in all sorts of laboratory investigations or administrative work which, as a rule, calls not for medical knowledge as such but for being conversant with the latest medical problems and a certain organizing ability. Our country's 92 medical educational establishments fail to meet the practical requirements of our public health system. As for institutes, faculties or even specialized secondary schools training hospital managerial personnel, our country has regrettably none as yet.

Another point I'd like to make is that seeking to train as many doctors as possible, we have markedly lowered the professional standards normally set for them. The emphasis on narrow specialization, lack of practical instruction in bedside care, and tight supervision of the newly qualified doctor's work have detracted greatly from the authority and prestige of the medical profession. It is no accident, therefore, that the problem of training general practitioners, the so called "family doctors," has now come to the fore. I think we would be

well advised to restore the undeservedly forgotten institution of general practice quite common in old Russia—but in an entirely new quality, of course.

N.T. This seems to confirm, in its own small way, that history often repeats itself. I recall Mikhail Bulgakov's story, "The Steel Throat," about a young doctor, fresh from college, doing a successful tracheotomy on a sick girl and saving her life. Is a typical budding doctor capable of performing such an operation today?

M. K. Diphtheria, which calls for such an operation, is no longer common, and a medical college graduate should not necessarily know how to perform one. Nevertheless, the situation is rather distressing: only a few of our college graduates are capable of performing even the simplest operations such as appendectomy. And mind you, the incidence of appendicitis is high today. This brings up, again, the question of whether narrow specialization is good or evil. To my mind, a young man who has completed a course in postgraduate clinical studies should be qualified in several specialties. As it is now, our district therapist is actually a dispatcher, his functions reduced to sending patients to specialists whom our polyclinics are always short of. A modern medical college graduate has vast theoretical knowledge, there is no denying that, but neither he nor society benefits from it. Theoretical knowledge is a far cry from practical skill. A family doctor is, in my opinion, supposed to be a harmonious combination of both.

N. T. A doctor can hardly confine himself to special theoretical knowledge and practical skill. He must be humane in the first place. You can't teach humanism, and knowledge is no substitute for it. How are those callous to the sufferings of others to be barred from the medical profession? Are medical college applicants to be tested for the sense of compassion?

M. K. We have repeatedly asked ourselves this question. Back in the sixties, when I was Rector of the First Moscow Medical Institute, we set up a laboratory to test applicants for entry and students for specific personality traits qualifying one to be a doctor. Years of observations and testing certainly helped us reveal definite tendencies, but we never succeeded in developing a technique of finding out whether a person is compassionate or not by nature. This is hardly possible at all.

I think that the extent to which a doctor meets the demands made on him by his day-to-day work—readiness to help his patients, to make every physical and moral effort so as to bring them back to health—is the best test for compassion. This test works unfailingly: the weak, the cruel and the heartless drop out. And medicine is none the worse for that, rather the contrary! Nevertheless, we can and must inculcate compassion in our students. This is to become part and parcel of instruction. Much depends here on the teacher's (and, later, a senior colleague's) personal example. Students are likely to learn by example, and if it is bad, they will take it for granted and act accordingly. Compassion springs from contact with the patient and largely depends on the doctor's personality. Pirogov would remind his students that if a patient doesn't feel any better after a talk with his doctor, the doctor is useless.

N. T. This probably explains why, for want of compassion, people have been chasing prestigious doctors of late. If surgery is prescribed, a patient goes all out to be operated upon by a "first-rate specialist," pinning his hopes solely on the name of a well-known surgeon, cardiologist or ophthalmologist. Does this "prudence" always make sense? Is the gap between the average professional standard in, say, surgery and that of a "celebrity" really so striking? What would be your expert comment on that?

M. K. It is only too natural that a patient should want a guaranteed cure. This is why he insists on being treated—even for a hernia—by the "first-rate specialist." With very rare exceptions, such "over-cautious" patients are merely egoistical—they distract a doctor from really grave cases and put him under nervous strain. Incidentally, more often than not people tend to settle for the services of a clinic or an institute enjoying a high reputation.

N. T. Could the problem of stepping up the public health service's efficiency and reducing the in-patient treatment period be solved by adopting, in general surgical practice, the so-called production-line method used by Professor Svyatoslav Fyodorov at his Moscow Ophthalmic Microsurgery Institute? True, this principle may further estrange the doctor from the patient and make the latter just an impersonal "object of treatment"...

M. K. Let me make it clear that "conveyer-belt" operations can be performed only on identical cases. Although he is not without his problems, Fyodorov has succeeded in "conveyerizing" eye surgery to a certain extent owing to the fact that the anatomical structure and position of the eye differ but little from patient to patient; consequently, crystalline lens defects can be corrected using strictly definite standard techniques. On the other hand, if you take appendectomy, for instance, the anatomical position of the appendix varies to a much greater extent, each case of appendicitis requiring an individual nonstandard approach. And what about stomach troubles or cancer of the rectum? You can't use any uniform surgical

techniques in these cases at all, let alone the "production-line" method—not only from the technical viewpoint, but from that of humanity and compassion as well.

In any case, a surgeon must take an individual approach to each particular patient, take his physiological and psychological condition into account, select the most humane mode of operative intervention, and painstakingly provide against any relapses. All this can be achieved, in my opinion, not through "conveyerization," but through the continuous improvement of surgical instruments and methods, through a better organization of our work and, naturally, through a steady rise in our entire hospital personnel's skill. Our medical science and industry can play a no small role here.

N. T. You have actually mentioned the basic problems of Soviet medicine, surgery in particular, now being tackled in this country in earnest. Nevertheless, the situation still leaves much to be desired. As far as science is concerned, some physicians are of the opinion that the Medical Academy has ceased to be the headquarters of medical research. Is that so? Haven't the changes now under way in the public health system found their reflection in the activity of the Academy of Medical Sciences?

M. K. It is my conviction that the Academy has been, remains and will be the headquarters of our science. I consider it an indisputable fact that our public health system owes all its substantial achievements to the Academy, above all.

It was under the immediate guidance of the members of the Academy working in its Moscow, Siberian and Uzbek branches that recommendations were worked out for the treatment of a terrible disease like the viral hepatitis B. By virtue of its specific nature, the Academy has a limited contingent of patients to assess its research findings, so how can it possibly answer for the state of public health on a national scale? Besides, having no administrative powers, the Academy of Medical Sciences is in no position to have even the simplest and the most effective methods of diagnosing and treating a particular disease put into common clinical use.

As I have already pointed out to you, new, effective and easy-to-use methods of treatment are often ignored even in Moscow, to say nothing of the periphery, because adopting them involves changes, even though slight, in the habitual order of things... The reason why our health system, both in Moscow and in other parts of the country, is way below standard lies in its failure to make adequate use of the Academy's scientific and practical potential.

N. T. Still, one can hardly describe as normal a situation where the Academy of Medical Sciences of the U.S.S.R. works in isolation from the public health service as a whole. After all, you can't do fundamental research for its own sake...

M. K. I beg to disagree. We at the Academy realize our difficulties full well, of course, and are taking steps to cope with them. For instance, we propose to streamline our research network, close down small and inefficient institutions and establish larger ones in their place.

Other avenues of our activity include the intensification of research, the promotion of inter-institute cooperation, and the extended reproduction of test animals. I shall add to this that we are going to make a practice of inviting orders for specific research and experimental jobs from the Ministry of Public Health, the U.S.S.R. State Committee for Science and Technology and related departments, and placing them with our research institutions on a competitive basis. This will guarantee the high quality of our research efforts. Translating this idea into reality is a common objective of all public health workers, from rank-and-file doctors to the heads of large clinics or research centers.

N. T. The self-financing now being introduced in the public health services will be a good stimulus to overall structural reforms and local initiative, won't it? On the other hand, would it be expedient for the entire public health system to go self-financing?

M. K. If you mean the principle of social justice, it will be in no way violated in this case; rather on the contrary: the technological, research and personnel bases of free medical service will be further consolidated by extra state funding. As for the introduction of paying medical services, I believe this to be absolutely necessary in certain cases. The treatment of occupational diseases, for instance, should be paid for not by district polyclinics, but by industrial plants. Incidentally, this is stipulated in the Basic Guidelines on the Development of the Public Health System. This will make every factory manager weigh what suits him better: introducing new equipment, improving safety engineering and making working conditions healthier, or showing no regard for his employees' requirements and health and regularly paying out large sums for medical treatment as a result.

Further, we can now offer patients special diets at their own expense. A network of cooperative in- and outpatient clinics will grow. However, it is not enough to introduce the mechanism of self-support; it should be continuously improved. For instance, hospitals can now afford to spend more on food for their patients, but they must get it only from definite supply centers whose choice of provisions is inadequate. Why can't a hospital arrange for a collective farm—or a nearby restaurant—to keep it supplied with food? Why can't a hospital purchase fruit and vegetables right at city markets?

N. T. The future is often unpredictable, but present-day trends may give us some insight into it. What do you think surgery will be like in the 21st century?

M. K. "We are no prophets...," a poet said. But one doesn't have to be a prophet to predict that if we go by the logic of science and facts the future of surgery lies in conservative treatment. This will be made possible by extensive prophylactic work, a healthier way of life, and healthier work conditions. The number of complicated operations will diminish sharply thanks to a more efficient early diagnosis system and timely disease prevention measures (surgical ones included) at the early stages of stomach, lung and mammary gland cancer, for instance. New methods of laboratory and instrumental diagnosis will become common to rule out mistakes in recognizing a disease and choosing the course of treating it. Universal prophylactic medical examination practice, which has already been initiated in our country and which I'd call a national public health care service, will go a long way towards making all this a reality. And I am confident that the 21st century surgeon will use the scalpel much less frequently than we do now.

UDC 577,391;591.862:615

Mechanism of Action of Stimulatory Effect of Regenerating Muscle Tissue on Recovery of Irradiated Skeletal Muscles

18400466a Moscow DOKLADY AKADEMII NAUK SSSR in Russian Vol 300 No 4, Jun 88 (manuscript received 4 Nov 87) pp 977-980

[Article by M. F. Popova and V. S. Azarova, Institute of Evolutionary Morphology and Ecology of Animals, USSR Academy of Sciences, Moscow]

[Abstract] Ionizing radiation has been demonstrated to diminish post-traumatic regeneration of the skeletal musculature, a phenomenon subject to reversal by the autotransplantation of minced skeletal muscle tissue. In order to assess the mechanism of action of the transplanted tissue on the regenerative process following trauma and irradiation, experiments were conducted on outbred albino rats with amputation stumps. The stumps were subjected to ionizing irradiation in a dose of 20 Gy followed by autotransplantation, with the transplant separated from the injured muscular tissue by either a cellophane or Synpor 8 membrane (pore size 0.22 μm) to evaluate the significance of diffusible factors on regeneration. In the cellophane membrane experiment, diffusion was prevented by the impermeable membrane, and both light and electron microscopy confirmed extensive atrophy and replacement of the distal stump by fibrous tissue over a two week period. However, in the Synpor 8 experiment considerable evidence of regeneration of the striated muscular tissue was obtained, which was attributed to the diffusion of metabolites from the minced tissue to the site of injury. The extent of recovery, however, in the two week period with the Synpor 8 membrane was not as pronounced as in experiments without a barrier between the transplant and the injured muscle, indicating the importance of contact phenomena in transplant-mediated post-traumatic recovery. Figures 4; references 15: 8 Russian, 7 Western.

UDC 591: 81.87

Effects of Regenerating Gastrocnemius Muscle on Splenocyte Radiosensitivity in C57B1 Mice

18400466c Moscow DOKLADY AKADEMII NAUK SSSR in Russian Vol 300 No 4, Jun 88 (manuscript received 5 Nov 87) pp 995-998

[Article by N. S. Samokhvalova, M. F. Popova and N. S. Kustova, Institute of Evolutionary Morphology and Ecology of Animals, USSR Academy of Sciences, Moscow]

[Abstract] Therapeutic trials were conducted in male and female C57B1 mice (18-25 g) to further assess the radioprotective effects of subcutaneous autotransplantation of minced gastrocnemius muscle in the vicinity of the spleen 10 days prior to 3.5 Gy whole body x-irradiation. A series of experiments was designed which included groups of animals with homotopic and heterotopic (spleen site) autotransplantation 10 days prior to irradiation, with the results evaluated in terms of chromosomal aberrations in splenocytes, their mitotic activity, viability, cellular depopulation of the spleen and splenic weight. Autotransplantation alone, either homo- or heterotopic, led to an increase in the number of chromosomal aberrations, in cell death, and in the mitotic index, concomitantly with a relative increase in splenic weight. Irradiation (whole body 3.5 Gy, 0.48 Gy/min) similarly resulted in significant splenic damage. A combination of homotopic transplantation and irradiation actually exacerbated the adverse effects on the spleen, while heterotopic transplantation was observed to mitigate subsequent radiation injury in the spleen to a statistically significant degree. The reduction in splenocyte damage in the 6 h postradiation period by the regenerating muscle tissue when transplanted in the vicinity of the spleen was attributed to activation of repair processes. Figures 1; references 13: 11 Russian, 2 Western.

UDC 577.391;612.386.86

Radioprotective Properties of Oxolinic Acid in Prolonged Radiation

18400479g Moscow RADIOBIOLOGIYA in Russian Vol 28 No 2, Mar-Apr 88 (manuscript received 2 Jun 87) pp 279-281

[Article by V. V. Pukhov and S. B. Daniyarov, Kirghiz State Medical Institute, Frunze]

[Abstract] Trials were conducted with 20-25 g outbred albino mice to assess the radioprotective properties of oxolinic acid in prolonged (6-8 h) 7.5-9.5 Gy gamma irradiation. Evaluation of the 300 day survival figures yielded survival rates of 7-33 percent, with the highest survival figures obtained with subcutaneous administration of 200 mg/kg of oxolinic acid. Intragastric administration was completely ineffective. The effectiveness of oxolinic acid was attributed to the depression of DNA synthesis in bone marrow karyocytes, which was most pronounced within 3 h of oxolinic acid administration and persisted for 24 h. Tables 2; references 6: 5 Russian, 1 Western.

UDC 577.391;615.739.16;621.386.86

Radioprotective Effects of Selected Nucleosides and Nucleotides and Mechanism of Action of Adenosine

18400479a Moscow RADIOBIOLOGIYA in Russian Vol 28 No 2, Mar-Apr 88 (manuscript received 8 Jul 88) pp 230-235

[Article by V. I. Kulinskiy, A. D. Klimova and I. V. Filippovich, Krasnoyarsk Medical Institute; Institute of Biophysics, USSR Academy of Sciences, Moscow]

[Abstract] A study was conducted on nucleosides and nucleotides to determine agents with potential radioprotective activities, employing 3-5 month old (CBA x

C57B1)F_1 female mice. The animals were x-irradiated with a 8.5 Gy dose 30 min after subcutaneous administration of the test agent (1.12 mmoles/kg, equimolar to 300 mg/kg adenosine). Adenosine and the majority of adenine mononucleotides were found to be effective radioprotectors. Adenine and 2'-deoxyadenosine failed to protect the mice, while 3', 5'-cAMP was weakly protective. Furthermore, ribo- and deoxyribonucleosides and nucleotides of guanine, uracil, thymine, and cytosine failed to exhibit radioprotection. In order to assess the mechanism of action of adenosine, additional studies involved subcutaenous administration of dipiridamol (5-40 mg/kg) or of an alkylxanthine (theophylline, caffeine, isobutyl methylxanthine) 40-45 min before irradiation. The fact that dipiridamol enhanced the radioprotective effectiveness of adenosine and the alkylxanthines blocked it demonstrated that the mechanism of action involved the interaction of adenosine with an A-receptor on the surface of the plasma membranes. Additional studies shall be required to define the subtype of the A-receptor involved in the radioprotective action of adenosine. Tables 2; references 27: 12 Russian, 15 Western.

UDC 577.391;591.818;612.015.32

Status of Lipid Phase of Biomembranes and Compensatory Reserves of Cell Energetic System in Irradiated Hypokinetic Animals

18400479b Moscow RADIOBIOLOGIYA in Russian Vol 28 No 2, Mar-Apr 88 (manuscript received 24 Jun 87) pp 245-249

[Article by V. I. Ivanov and A. A. Turdyyev, Institute of Biochemistry, Uzbek SSR Academy of Sciences, Tashkent]

[Abstract] Previous studies have shown that hypokinesia enhanced lipid peroxidation, a physiologic change that in turn increases radiosusceptibility. To define the mechanism involved in the interaction of hypokinesia and irradiation, outbred male rats (180-206 g) were subjected to limited mobility for two weeks in a plexiglass container, gamma irradiated (8 Gy) during that time, and maintained under hypokinetic conditions for another week prior to biochemical studies. The experiments demonstrated that irradiation of intact rats led to an increase in the concentration of diene ketones in hepatic mitochondria to a level exceeding 1.5-fold that of control rats 7 days after irradiation. The levels of conjugated dienes in the mitochondria and microsomes increased 1.3- and 1.2-fold, respectively. The combination of hypokinesia and irradiation resulted in more pronounced increases which, furthermore, were more pronounced in the case of the hepatic mitochondria than in the microsomes. Combined hypokinesia and irradiation enhanced use of the compensatory reserve of the mitochondrial oxidative chain by 242 percent, versus a figure of 126 percent for hypokinesia alone and a figure of 233 percent with irradiation as the sole factor. Activities of ATPase and succinic dehydrogenase fell by an average of 27 percent, confirming a compensated low-energy shift. Radiation alone led to an increase in the cholesterol:phospholipid molar ratio in mitochondria and microsomes, while a combination of hypokinesia and irradiation led to depressed ratios. Thus, while irradiation remained the primary pathogenic factor, hypokinesia acted to potentiate the sequelae of irradiation through its effects on the lipid phase of the organelles and cellular energetics. Figures 2; tables 4; references 13 (Russian).

UDC 577.391;631.531.1

Electrophoretic Analysis of Isoenzymes Associated with Radioresistance of Hexaploid Wheat Seeds

18400479c Moscow RADIOBIOLOGIYA in Russian Vol 28 No 2, Mar-Apr 88 (manuscript received 25 Sep 87) pp 250-255

[Article by O. P. Yumasheva, B. I. Sarapultsev and R. M. Aleksakhin, All-Union Scientific Research Institute of Agricultural Radiology, USSR Gosagroprom, Obninsk]

[Abstract] An electrophoretic analysis was conducted on the isoenzyme patterns of a series of enzymes on two groups of hexaploid wheat (T. aestivum) markedly differing in radioresistance. Analysis of the patterns obtained with polyacrylamide gel electrophoresis for seeds and shoots revealed highly specific differences between susceptible and resistant varieties in the case of malate dehydrogenase (MDH) and leucine aminopeptidase (LAP). The data applied particularly to the components MDH^9, MDH^{10}, LAP^2, and LAP^5. The findings were consistent with the view that genes Mdh^9, Mdh^{10}, Lap^2, and Lap^5 are linked to systems responsible for genetic control of radioresistance and are located on chromosomes 1A, 1B, and 6A. Figures 2; references 18: 4 Russian, 14 Western.

UDC 577.391;621.375.8;576.8

Effects of Laser and Combined Alpha and Laser Radiation on Lag Time in Bacterial Division

18400479d Moscow RADIOBIOLOGIYA in Russian Vol 28 No 2, Mar-Apr 88 (manuscript received 29 Jul 87) pp 262-264

[Article by N. V. Simonyan and K. Sh. Voskanyan, Yerevan Physical Institute, State Committee on Atomic Energy; Scientific Research Institute of Condensed Media Physics, Yerevan State University]

[Abstract] In view of the suggestion that the radioprotective effects of laser illumination on bacterial systems are due to a prolonged lag phase, i.e., an increase in the time available for repair mechanisms to take place, the hypothesis was tested on Escherichia coli K-12 strain AB 1157 cells subjected to laser illumination alone, and to a laser + alpha-irradiation combination. On transfer to a new medium the E. coli cells commenced division after a lag time of 1.5 h. Cells subjected to a continuous emission from a helium-neon laser (633 nm, 8 x 10^3

J/m^2) for 5 sec also showed a 1.5 h lag time, while cells illuminated for 12 sec exhibited a lag time of 2.5 h. Alpha-irradiation (210 Gy; 13 Gy/min) of the E. coli cells before or after laser irradiation also yielded 2.5 h lag times. These findings indicated, therefore, that the radioprotective effects of laser irradiation are unrelated to postradiation delay in the lag time. Figures 2; references 4 (Russian).

UDC 577.391;599.323.4

Effects of Ionizing Radiation on Aggressive Behavior of Mice

18400479e Moscow RADIOBIOLOGIYA in Russian Vol 28 No 2, Mar-Apr 88 (manuscript received 23 Apr 87) pp 264-268

[Article by A. A. Mogilner, Scientific Research Institute of Medical Radiology, USSR Academy of Medical Sciences, Obninsk]

[Abstract] In view of the virtual lack of studies on the effects of ionizing radiation on fine psychophysiological processes, studies were undertaken on outbred male mice to determine the effects of gamma-irradiation on intraspecies aggression. Evaluation of the attack frequencies 3-4 and 24 h after irradiation demonstrated that dosages of 5, 10, and 30 Gy were without behavioral sequelae. However, dosages of 60 and 100 Gy markedly attenuated aggressive patterns of behavior in cases of individual as well as group irradiation. In addition, stress in the form of discomfort at the time of irradiation enhanced the antiaggressive effects of gamma-irradiation. Figures 2; tables 2, references 5 (Russian).

UDC 577.391;599.323.4;621.386.86

Radioprotective Effects of Quinoline Derivatives

18400479f Moscow RADIOBIOLOGIYA in Russian Vol 28 No 2, Mar-Apr 88 (manuscript received 22 Sep 87) pp 274-276

[Article by M. V. Vasin, N. N. Suvorov, L. A. Semenova and G. N. Ilyina]

[Abstract] A total of 12 derivatives of quinoline were evaluated for radioprotective activity on dihybrid (CBA x C57B1/6)F_1 and tetrahybrid (CBWA)F_2 mice, 21-26 g, subjected to gamma irradiation in doses of 9.5 and 9.0 Gy, respectively. Evaluations were based on 30 day survival figures, with the animals treated with the agents 5-20 min before irradiation as intraperitoneal injections prepared ex tempore (0.2 ml/mouse). Three of the derivatives—1-(2-quinolyl)piperazine maleate, 1-(2-quinolyl)-4-(carbethoxymethyl)piperazine, and 8-(1-piperazine)-1-H-pyrrolo[3,2-h]quinoline—offered protection at the 50 percent level. In addition, hydrazides of piperazinylcinchonine offered radioprotective effects at the 70 percent level, i.e., essentially equivalent to the protective effects of mexamine. Tables 1; references 3: 2 Russian, 1 Western.

UDC 576.8

Inhibition of Adenovirus Replication in Cos I Cell Culture by Antisense RNA

18400466b Moscow DOKLADY AKADEMII NAUK SSSR in Russian Vol 300 No 4, Jun 88 (manuscript received 7 Dec 87) pp 981-982

[Article by O. I. Miroshnichenko, T. I. Ponomareva and T. I. Tikhonenko, All-Union Scientific Research Institute of Agricultural Biotechnology, All-Union Order of Lenin Academy of Agricultural Sciences imeni V. I. Lenin (VASKhNIL), Moscow]

[Abstract] Cursory details are presented on genetic engineering studies employed in studies on the effects of antisense RNA (asRNA) on the reproduction of type 5 adenovirus in cultured monkey Cos I cells. The asRNA was targeted against the EIA region of the adenoviral genome, which has a key role in the regulation of other adenoviral genes. A recombinant vector designated ppS-NEO containing Moloney leukemia genome was employed in the synthesis of asRNA, as well as recombinant plasmid ppA in which the EIA region was inverted in its orientation to the Moloney promoter. In the system under study the asRNA was found to be an efficient inhibitor of type 5 adenovirus replication. These observations may serve as a stepping stone toward the creation of more efficient systems for the inhibition of adenovirus replication with potential clinical implications. Tables 1; references 15 (Western).

UDC 576.316.7.085.23:578.833.26

Cytogenetic and Virologic Characteristics of an RH Cell Line and Its Clones with Different Sensitivity to Tick-Borne Encephalitis Virus

18400512a Leningrad TSITOLOGIYA in Russian Vol 30 No 4, Apr 88 (manuscript received 6 Apr 87) pp 454-459

[Article by N. A. Popova, S. Ye. Mamayeva, M. A. Arkina, T. L. Miryutova, and L. M. Laptakova, Scientific Research Institute of Vaccines and Sera, USSR Ministry of Health, Tomsk; Institute of Cytology, USSR Academy of Sciences, Leningrad]

[Abstract] In most cell cultures propagation of tick-borne encephalitis (TBE) virus is not accompanied by any cytopathic changes; only in pig embryonic kidney cell cultures does TBE lead to a clear cytopathic effect. Therefore, it is of great interest to develop new cell models which are capable of cell destruction in TBE virus reproduction and can be used as test systems in determining the activity of TBE virus. It was noted that the RH line from HeLa cells exhibited such sensitivity to TBE virus. The goal of this work was to clone cell populations from the RH line with different sensitivities to TBE virus and to characterize them and the RH line karyologically. Virologic and cytogenetic characterization of two clones (RH k-20 and RH k-13/6) with different sensitivities to the virus were given: the RH k-13/6 cells were 10^4 times as sensitive as the RH k-20 cells. Karyologic study of the cell line and the clones indicated a stabilization of karyotypes: RH k-20 and the starting line had 65 chromosomes; the RH k-13/6 line, 66 chromosomes. All the cells were hypotriploid. The sensitive clone RH k-13/6 exhibited a third homologue of chromosome 8, not present in the starting line or in the RH k-20. Figure 1; references 21: 5 Russian, 16 Western.